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 * * * * * * * * * * * * * STN Columbus * * * * * * * * * * * * * * *
=> file biosis,caba,caplus,embase,japio,lifesci,medline,scisearch,uspatfull
=> e mitchell william m/au
E1        3   MITCHELL WILLIAM LUNSFORD/AU
E2        1   MITCHELL WILLIAM LUNSFORD III/AU
E3      124 --> MITCHELL WILLIAM M/AU
E4        3   MITCHELL WILLIAM MARK/AU
E5        4   MITCHELL WILLIAM MARVIN/AU
E6        1   MITCHELL WILLIAM MORRIS/AU
E7        1   MITCHELL WILLIAM N/AU
E8       22   MITCHELL WILLIAM O/AU
E9        4   MITCHELL WILLIAM P/AU
E10       9   MITCHELL WILLIAM R/AU
E11       2   MITCHELL WILLIAM RICHARD/AU
E12       1   MITCHELL WILLIAM ROBERTSON/AU
=> s e3-e6 and chlamydia
L1       27 ("MITCHELL WILLIAM M"/AU OR "MITCHELL WILLIAM MARK"/AU OR "MITCH
           ELL WILLIAM MARVIN"/AU OR "MITCHELL WILLIAM MORRIS"/AU) AND
           CHLAMYDIA
=> dup rem l1
PROCESSING COMPLETED FOR L1
L2       21 DUP REM L1 (6 DUPLICATES REMOVED)
=> d 1-
YOU HAVE REQUESTED DATA FROM 21 ANSWERS - CONTINUE? Y/(N):y
 L2  ANSWER 1 OF 21  BIOSIS  COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
    DUPLICATE 1
AN  2003:323691  BIOSIS
DN  PREV200300323691
TI  Diagnosis and management of infection caused by  ***chlamydia***  .
AU    ***Mitchell, William M.***  [Inventor, Reprint Author]; Stratton,
    Charles W. [Inventor]
CS  ASSIGNEE: Vanderbilt University
PI  US 6579854 June 17, 2003
SO  Official Gazette of the United States Patent and Trademark Office Patents,
    (June 17, 2003) Vol. 1271, No. 3. http://www.uspto.gov/web/menu/patdata.ht
    ml. e-file.
    ISSN: 0098-1133 (ISSN print).
DT  Patent
LA  English
ED  Entered STN: 9 Jul 2003
    Last Updated on STN: 9 Jul 2003

L2  ANSWER 2 OF 21  USPATFULL on STN
AN    2003:318218  USPATFULL
TI    Methods and reagents for the treatment of multiple sclerosis
IN    Stratton, Charles W., Nashville, TN, UNITED STATES
        ***Mitchell, William M.***  , Nashville, TN, UNITED STATES
      Sriram, Subramaniam, Nashville, TN, UNITED STATES
PI    US 2003223959     A1   20031204
AI    US 2003-419034    A1   20030417 (10)
RLI   Continuation of Ser. No. US 2000-528348, filed on 17 Mar 2000, PENDING
      Continuation-in-part of Ser. No. US 1998-73661, filed on 6 May 1998,
      GRANTED, Pat. No. US 6579854 Continuation-in-part of Ser. No. US
      1998-25174, filed on 18 Feb 1998, GRANTED, Pat. No. US 6562582
      Continuation-in-part of Ser. No. US 1997-911593, filed on 14 Aug 1997,
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ABANDONED
PRAI US 1999-125598P 19990319 (60)
US 2000-176662P 20000118 (60)
US 2000-176940P 20000118 (60)
US 2000-176784P 20000118 (60)
DT Utility
FS APPLICATION
LN.CNT 2445
INCL INCLM: 424/085.400
INCLS: 514/028.000; 514/291.000; 514/252.130; 514/192.000; 514/253.080; 514/312.000; 514/152.000; 514/602.000
NCL NCLM: 424/085.400
NCLS: 514/028.000; 514/291.000; 514/252.130; 514/192.000; 514/253.080; 514/312.000; 514/152.000; 514/602.000
IC [7]
ICM: A61K038-21
ICS: A61K031-7048; A61K031-496; A61K031-65; A61K031-18; A61K031-4706
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L2 ANSWER 3 OF 21 USPATFULL on STN
AN 2003:277157 USPATFULL
TI Diagnosis and management of infection caused by ***chlamydia***
IN ***Mitchell, William M.*** , Nashville, TN, UNITED STATES
Stratton, Charles W., Nashville, TN, UNITED STATES
PI US 2003195184 A1 20031016
AI US 2002-101279 A1 20020319 (10)
RLI Continuation of Ser. No. US 1998-73661, filed on 6 May 1998, GRANTED, Pat. No. US 6579854 Continuation-in-part of Ser. No. US 1998-25521, filed on 18 Feb 1998, ABANDONED Continuation-in-part of Ser. No. US 1997-911593, filed on 14 Aug 1997, ABANDONED Continuation-in-part of Ser. No. US 1998-73661, filed on 6 May 1998, GRANTED, Pat. No. US 6579854 Continuation-in-part of Ser. No. US 1998-25176, filed on 18 Feb 1998, GRANTED, Pat. No. US 6258532 Continuation-in-part of Ser. No. US 1997-911593, filed on 14 Aug 1997, ABANDONED
PRAI US 1997-45739P 19970506 (60)
US 1997-45779P 19970506 (60)
US 1997-45780P 19970506 (60)
US 1997-45784P 19970506 (60)
US 1997-45787P 19970506 (60)
US 1997-45689P 19970506 (60)
DT Utility
FS APPLICATION
LN.CNT 4849
INCL INCLM: 514/192.000
INCLS: 514/210.090; 514/398.000; 514/471.000; 514/562.000; 514/665.000; 514/574.000
NCL NCLM: 514/192.000
NCLS: 514/210.090; 514/398.000; 514/471.000; 514/562.000; 514/665.000; 514/574.000
IC [7]
ICM: A61K031-407
ICS: A61K031-397; A61K031-198; A61K031-4168; A61K031-19; A61K031-13
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L2 ANSWER 4 OF 21 USPATFULL on STN

AN 2003:250524 USPATFULL
TI Identification of antigenic peptide sequences
IN ***Mitchell, William M.*** , Nashville, TN, UNITED STATES
Stratton, Charles W., Nashville, TN, UNITED STATES
PI US 2003175310 A1 20030918
AI US 2001-20269 A1 20011214 (10)
RLI Continuation of Ser. No. US 1998-25596, filed on 18 Feb 1998, GRANTED, Pat. No. US 6340463 Continuation-in-part of Ser. No. US 1997-911593, filed on 14 Aug 1997, ABANDONED
PRAI US 1996-23921P 19960814 (60)
DT Utility
FS APPLICATION
LN.CNT 1728
INCL INCLM: 424/263.100
INCLS: 530/350.000; 530/388.400; 536/023.700
NCL NCLM: 424/263.100
NCLS: 530/350.000; 530/388.400; 536/023.700
IC [7]
ICM: A61K039-118
ICS: C07H021-04; C07K014-295; C07K016-12
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L2 ANSWER 5 OF 21 USPATFULL on STN
AN 2003:244934 USPATFULL
TI Diagnosis and management of infection caused by ***Chlamydia***
IN ***Mitchell, William M.*** , Nashville, TN, UNITED STATES
Stratton, Charles W., Nashville, TN, UNITED STATES
PI US 2003171348 A1 20030911
US 6664239 B2 20031216
AI US 2002-100785 A1 20020319 (10)
RLI Continuation of Ser. No. US 1998-73661, filed on 6 May 1998, PENDING Continuation-in-part of Ser. No. US 1998-25521, filed on 18 Feb 1998, ABANDONED Continuation-in-part of Ser. No. US 1997-911593, filed on 14 Aug 1997, ABANDONED
PRAI US 1997-45739P 19970506 (60)
US 1997-45779P 19970506 (60)
US 1997-45780P 19970506 (60)
US 1997-45784P 19970506 (60)
US 1997-45787P 19970506 (60)
US 1997-45689P 19970506 (60)
DT Utility
FS APPLICATION
LN.CNT 4871
INCL INCLM: 514/192.000
INCLS: 514/210.090; 514/398.000; 514/471.000; 514/562.000; 514/574.000; 514/665.000
NCL NCLM: 514/029.000
NCLS: 514/031.000; 514/152.000; 514/179.000; 514/192.000; 514/199.000; 514/311.000; 514/312.000; 514/601.000
IC [7]
ICM: A61K031-397
ICS: A61K031-407; A61K031-4168; A61K031-198; A61K031-19; A61K031-13
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L2 ANSWER 6 OF 21 USPATFULL on STN

AN 2003:268206 USPATFULL
TI Methods for inducing mucosal immune responses
IN ***Mitchell, William M.*** , Nashville, TN, United States
PA Vanderbilt University, Nashville, TN, United States (U.S. corporation)
PI US 6630455 B1 20031007
AI US 1995-372429 19950113 (8)
DT Utility
FS GRANTED
LN.CNT 2363
INCL INCLM: 514/044.000
INCLS: 435/440.000; 435/458.000
NCL NCLM: 514/044.000
NCLS: 435/440.000; 435/458.000
IC [7]
ICM: A01N043-04
ICS: A61K031-70; C12N015-00; C12N015-88
EXF 435/69.1; 435/172.3; 435/320.1; 435/172.1; 435/240.1; 435/440; 435/458; 424/200.1; 424/130.1; 424/184.1; 514/44; 514/2; 514/731
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L2 ANSWER 7 OF 21 MEDLINE on STN
AN 2003261989 MEDLINE
DN 22672019 PubMed ID: 12789196
TI Culture and immunohistochemical evidence of ***Chlamydia*** pneumoniae infection in ulcerative pyoderma gangrenosum.
AU Sams Hunter H; ***Mitchell William M*** ; Stratton Charles W; King Lloyd E Jr
CS Department of Medicine, Vanderbilt University Medical Center, Nashville, Tennesses, USA.
SO JOURNAL OF THE AMERICAN ACADEMY OF DERMATOLOGY, (2003 Jun) 48 (6) 966-9. Journal code: 7907132. ISSN: 0190-9622.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 200307
ED Entered STN: 20030606
Last Updated on STN: 20030715
Entered Medline: 20030714

L2 ANSWER 8 OF 21 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
AN 2003:201067 BIOSIS
DN PREV200300201067
TI Qualitative and quantitative detection of ***chlamydia*** pneumoniae DNA in cerebrospinal fluid.
AU Sriram, Subramaniam [Reprint Author]; Yao, Song-yi [Reprint Author]; Li, Haijing [Reprint Author]; Stratton, Charles [Reprint Author]; ***Mitchell, William M.*** [Reprint Author]; Meng, Shufang [Reprint Author]; Tang, Yi-Wei [Reprint Author]
CS Nashville, TN, USA
SO Neurology, (March 11, 2003) Vol. 60, No. 5 Supplement 1, pp. A399. print. Meeting Info.: 55th Annual Meeting of the American Academy of Neurology. Honolulu, Hawaii, USA. March 29-April 05, 2003. ISSN: 0028-3878 (ISSN print).
DT Conference; (Meeting)

Conference; Abstract; (Meeting Abstract)
LA English
ED Entered STN: 23 Apr 2003
Last Updated on STN: 23 Apr 2003

L2 ANSWER 9 OF 21 CAPLUS COPYRIGHT 2004 ACS on STN DUPLICATE 2
AN 2002:65982 CAPLUS
DN 136:133602
TI Identification of antigenic peptide sequences
IN ***Mitchell, William M.*** ; Stratton, Charles W.
PA Vanderbilt University, USA
SO U.S., 40 pp., Cont.-in-part of U.S. Ser. No. 911,593, abandoned.
CODEN: USXXAM
DT Patent
LA English
FAN.CNT 6

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI US 6340463	B1	20020122	US 1998-25596	19980218
US 2003175310	A1	20030918	US 2001-20269	20011214

PRAI US 1996-23921P P 19960814
US 1997-911593 B2 19970814
US 1998-25596 A1 19980218
RE.CNT 7 THERE ARE 7 CITED REFERENCES AVAILABLE FOR THIS RECORD
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L2 ANSWER 10 OF 21 USPATFULL on STN
AN 2002:16922 USPATFULL
TI ***CHLAMYDIA*** -FREE CELL LINES AND ANIMALS
IN ***MITCHELL, WILLIAM M.*** , NASHVILLE, TN, UNITED STATES
STRATTON, CHARLES W., NASHVILLE, TN, UNITED STATES
PI US 2002009802 A1 20020124
US 6562582 B2 20030513
AI US 1998-25174 A1 19980218 (9)
RLI Continuation of Ser. No. US 1997-911593, filed on 14 Aug 1997, ABANDONED
DT Utility
FS APPLICATION
LN.CNT 676
INCL INCLM: 435/325.000
INCLS: 435/031.000; 435/384.000
NCL NCLM: 435/032.000
NCLS: 424/009.100; 424/405.000; 435/031.000; 435/325.000; 435/366.000;
800/014.000
IC [7]
ICM: C12N005-00
ICS: C12N005-02; C12Q001-22
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L2 ANSWER 11 OF 21 CAPLUS COPYRIGHT 2004 ACS on STN DUPLICATE 3
AN 2001:397834 CAPLUS
DN 135:2559
TI Methods for in vitro susceptibility testing of ***Chlamydia***
IN Stratton, Charles W.; ***Mitchell, William M.***
PA USA
SO U.S. Pat. Appl. Publ., 9 pp., Cont.-in-part of U.S. Ser. No. 911,593,

abandoned.
CODEN: USXXCO
DT Patent
LA English
FAN.CNT 6

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI US 2001002421	A1	20010531	US 1998-25176	19980218
US 6258532	B1	20010710		
WO 9850074	A2	19981112	WO 1998-US9237	19980506
WO 9850074	A3	19990819		

W: AL, AM, AT, AU, AZ, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, GW, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM

RW: GH, GM, KE, LS, MW, SD, SZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG

AU 9872899	A1	19981127	AU 1998-72899	19980506
AU 746381	B2	20020418		
EP 981372	A2	20000301	EP 1998-920292	19980506

R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, FI

JP 2002512622	T2	20020423	JP 1998-548440	19980506
US 6579854	B1	20030617	US 1998-73661	19980506
US 2003195184	A1	20031016	US 2002-101279	20020319
PRAI US 1997-911593	B2	19970814		
US 1996-23921P	P	19960814		
US 1997-45689P	P	19970506		
US 1997-45739P	P	19970506		
US 1997-45779P	P	19970506		
US 1997-45780P	P	19970506		
US 1997-45784P	P	19970506		
US 1997-45787P	P	19970506		
US 1998-25174	A	19980218		
US 1998-25176	A2	19980218		
US 1998-25521	A2	19980218		
US 1998-73661	A1	19980506		
WO 1998-US9237	W	19980506		

L2 ANSWER 12 OF 21 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
AN 2001:387345 BIOSIS
DN PREV200100387345
TI Methods for in vitro susceptibility testing of ***chlamydia*** .
AU Stratton, Charles W. [Inventor, Reprint author]; ***Mitchell, William*** *** M.*** [Inventor]
CS Nashville, TN, USA
ASSIGNEE: Vanderbilt University
PI US 6258532 July 10, 2001
SO Official Gazette of the United States Patent and Trademark Office Patents, (July 10, 2001) Vol. 1248, No. 2. e-file.
CODEN: OGUPE7. ISSN: 0098-1133.
DT Patent
LA English

ED Entered STN: 15 Aug 2001
Last Updated on STN: 19 Feb 2002

L2 ANSWER 13 OF 21 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
DUPLICATE 4
AN 2001:210808 BIOSIS
DN PREV200100210808
TI Regulation by IFN-beta of inducible nitric oxide synthase and interleukin-12/p40 in murine macrophages cultured in the presence of ***Chlamydia*** pneumoniae antigens.
AU Yao, Song-Yi; Ljunggren-Rose, Asa; Stratton, Charles W.; ***Mitchell,*** *** William M.*** ; Sriram, Subramaniam [Reprint author]
CS Multiple Sclerosis Research Laboratory, Vanderbilt Stallworth Rehabilitation Hospital, 2201 Capers Avenue, 1222H, Nashville, TN, 37212, USA
srirams@ctrvax.vanderbilt.edu
SO Journal of Interferon and Cytokine Research, (March, 2001) Vol. 21, No. 3, pp. 137-146. print.
ISSN: 1079-9907.
DT Article
LA English
ED Entered STN: 2 May 2001
Last Updated on STN: 18 Feb 2002

L2 ANSWER 14 OF 21 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
AN 2001:362824 BIOSIS
DN PREV200100362824
TI Potential role of ***Chlamydia*** pneumoniae in the pathogenesis of interstitial cystitis.
AU Alberts, Gregory L. [Reprint author]; Stratton, Charles W. [Reprint author]; ***Mitchell, William M.*** [Reprint author]; Franke, Jenny J. [Reprint author]
CS Nashville, TN, USA
SO Journal of Urology, (May, 2001) Vol. 165, No. 5 Supplement, pp. 68. print.
Meeting Info.: Annual Meeting of the American Urological Association, Inc. Anaheim, California, USA. June 02-07, 2001. American Urological Association, Inc.
CODEN: JOURAA. ISSN: 0022-5347.
DT Conference; (Meeting)
Conference; Abstract; (Meeting Abstract)
LA English
ED Entered STN: 2 Aug 2001
Last Updated on STN: 19 Feb 2002

L2 ANSWER 15 OF 21 CAPLUS COPYRIGHT 2004 ACS on STN
AN 2000:688466 CAPLUS
DN 133:249334
TI Methods and reagents for the diagnosis and treatment of multiple sclerosis caused by ***Chlamydia***
IN Stratton, Charles W.; ***Mitchell, William M.*** ; Yao, Song-yi; Bannan, Jason D.; Ljunggren-Rose, Asa; Sriram, Subramaniam
PA Vanderbilt University, USA
SO PCT Int. Appl., 102 pp.
CODEN: PIXXD2
DT Patent

LA English
FAN.CNT 6
PATENT NO. KIND DATE APPLICATION NO. DATE
---------- ---- -------- ---------- --------
PI WO 2000057187 A2 20000928 WO 2000-US7226 20000317
WO 2000057187 A3 20010419
W: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM
RW: GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG
EP 1166117 A2 20020102 EP 2000-916513 20000317
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO
PRAI US 1999-125598P P 19990319
US 2000-176662P P 20000118
US 2000-176784P P 20000118
US 2000-176940P P 20000118
US 2000-528348 A 20000317
WO 2000-US7226 W 20000317

L2 ANSWER 16 OF 21 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
AN 2000:124996 BIOSIS
DN PREV200000124996
TI Pyoderma gangrenosum and ***Chlamydia*** pneumoniae infection in a diabetic man: Pathogenic role or coincidence?.
AU Vannucci, Stephen A.; ***Mitchell, William M.*** ; Stratton, Charles W.; King, Lloyd E., Jr. [Reprint author]
CS Division of Dermatology, Vanderbilt Clinic, Vanderbilt University, Suite 3900, Nashville, TN, 37232-5227, USA
SO Journal of the American Academy of Dermatology, (Feb., 2000) Vol. 42, No. 2 Part 1, pp. 295-297. print.
ISSN: 0190-9622.
DT Article
LA English
ED Entered STN: 5 Apr 2000
Last Updated on STN: 3 Jan 2002

L2 ANSWER 17 OF 21 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
AN 2000:221691 BIOSIS
DN PREV200000221691
TI Induction of IL-12/p40 and NO- by C. pneumoniae antigens in murine macrophages is regulated by beta interferon.
AU Sriram, Subramaniam [Reprint author]; Yao, Song-Yi [Reprint author]; Ljunggren-Rose, O. [Reprint author]; Stratton, Charles W. [Reprint author]; ***Mitchell, William M.*** [Reprint author]
CS Nashville, TN, USA
SO Neurology, (April 11, 2000) Vol. 54, No. 7 Supp. 3, pp. A166. print.
Meeting Info.: 52nd Annual Meeting of the American Academy of Neurology. San Diego, CA, USA. April 29-May 06, 2000. American Academy of Neurology.
CODEN: NEURAI. ISSN: 0028-3878.

DT Conference; (Meeting)
Conference; Abstract; (Meeting Abstract)
LA English
ED Entered STN: 31 May 2000
Last Updated on STN: 5 Jan 2002

L2 ANSWER 18 OF 21 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
AN 1999:160215 BIOSIS
DN PREV199900160215
TI ***Chlamydia*** pneumoniae in patients with interstitial cystitis.
AU Franke, Jenny J.; Stratton, Charles W.; ***Mitchell, William M.***
CS Nashville, TN, USA
SO Journal of Urology, (April, 1999) Vol. 161, No. 4 SUPPL., pp. 29. print.
Meeting Info.: 94th Annual Meeting of the American Urological Association, Inc. Dallas, Texas, USA. May 1-6, 1999. American Urological Association.
CODEN: JOURAA. ISSN: 0022-5347.
DT Conference; (Meeting)
Conference; Abstract; (Meeting Abstract)
LA English
ED Entered STN: 16 Apr 1999
Last Updated on STN: 16 Apr 1999

L2 ANSWER 19 OF 21 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
DUPLICATE 5
AN 1999:347842 BIOSIS
DN PREV199900347842
TI ***Chlamydia*** pneumoniae infection of the central nervous system in multiple sclerosis.
AU Sriram, Subramaniam [Reprint author]; Stratton, Charles W.; Yao, Song-yi; Tharp, Anthony; Ding, Lingmei; Bannan, Jason D.; ***Mitchell, William*** *** M.***
CS Multiple Sclerosis Research Laboratory, 1222H Vanderbilt Stallworth Rehabilitation Hospital, 2201 Capers Avenue, Nashville, TN, 37212, USA
SO Annals of Neurology, (July, 1999) Vol. 46, No. 1, pp. 6-14. print.
CODEN: ANNED3. ISSN: 0364-5134.
DT Article
LA English
ED Entered STN: 24 Aug 1999
Last Updated on STN: 24 Aug 1999

L2 ANSWER 20 OF 21 CAPLUS COPYRIGHT 2004 ACS on STN
AN 1998:752291 CAPLUS
DN 130:10609
TI Diagnosis and management of infection caused by ***Chlamydia***
IN ***Mitchell, William M.*** ; Stratton, Charles W.
PA Vanderbilt University, USA
SO PCT Int. Appl., 139 pp.
CODEN: PIXXD2
DT Patent
LA English
FAN.CNT 6

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI WO 9850074	A2	19981112	WO 1998-US9237	19980506
WO 9850074	A3	19990819		

W: AL, AM, AT, AU, AZ, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, GW, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM
RW: GH, GM, KE, LS, MW, SD, SZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG
US 2001002421 A1 20010531 US 1998-25176 19980218
US 6258532 B1 20010710
AU 9872899 A1 19981127 AU 1998-72899 19980506
AU 746381 B2 20020418
EP 981372 A2 20000301 EP 1998-920292 19980506
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, FI
ZA 9803798 A 20000307 ZA 1998-3798 19980506
JP 2002512622 T2 20020423 JP 1998-548440 19980506
PRAI US 1997-45689P P 19970506
US 1997-45739P P 19970506
US 1997-45779P P 19970506
US 1997-45780P P 19970506
US 1997-45784P P 19970506
US 1997-45787P P 19970506
US 1997-911593 A 19970814
US 1998-25176 A2 19980218
US 1998-25521 A2 19980218
US 1998-25174 A 19980218
WO 1998-US9237 W 19980506

L2 ANSWER 21 OF 21 CAPLUS COPYRIGHT 2004 ACS on STN
AN 1998:124043 CAPLUS
DN 128:201045
TI Compositions of antichlamydial agents for the diagnosis and management of infection caused by ***chlamydia***
IN ***Mitchell, William M.*** ; Stratton, Charles W.
PA Vanderbilt University, USA; Mitchell, William M.; Stratton, Charles W.
SO PCT Int. Appl., 83 pp.
CODEN: PIXXD2
DT Patent
LA English
FAN.CNT 6

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI WO 9806435	A2	19980219	WO 1997-US14402	19970814
WO 9806435	A3	19980409		

W: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM
RW: GH, KE, LS, MW, SD, SZ, UG, ZW, AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG
AU 9741516 A1 19980306 AU 1997-41516 19970814
PRAI US 1996-23921P P 19960814

WO 1997-US14402 W 19970814

=> e stratton charles w/au
E1 10 STRATTON CHARLES L/AU
E2 1 STRATTON CHARLES S/AU
E3 99 --> STRATTON CHARLES W/AU
E4 1 STRATTON CHARLES W IV/AU
E5 11 STRATTON CHARLOTTE D/AU
E6 1 STRATTON CHRISTOPHER LESLIE/AU
E7 4 STRATTON CLAUD L/AU
E8 2 STRATTON CLEO C/AU
E9 1 STRATTON CLIFFORD/AU
E10 6 STRATTON CLIFFORD J/AU
E11 2 STRATTON COREY J/AU
E12 1 STRATTON CRAIG A/AU

=> s e3-e4 and chlamydia
L3 30 ("STRATTON CHARLES W"/AU OR "STRATTON CHARLES W IV"/AU) AND CHLAMYDIA

=> dup rem l3
PROCESSING COMPLETED FOR L3
L4 22 DUP REM L3 (8 DUPLICATES REMOVED)

=> d 1-
YOU HAVE REQUESTED DATA FROM 22 ANSWERS - CONTINUE? Y/(N):y

L4 ANSWER 1 OF 22 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
DUPLICATE 1
AN 2003:323691 BIOSIS
DN PREV200300323691
TI Diagnosis and management of infection caused by ***chlamydia*** .
AU Mitchell, William M. [Inventor, Reprint Author]; ***Stratton, Charles***
*** W.*** [Inventor]
CS ASSIGNEE: Vanderbilt University
PI US 6579854 June 17, 2003
SO Official Gazette of the United States Patent and Trademark Office Patents,
(June 17, 2003) Vol. 1271, No. 3. http://www.uspto.gov/web/menu/patdata.ht
ml. e-file.
ISSN: 0098-1133 (ISSN print).
DT Patent
LA English
ED Entered STN: 9 Jul 2003
Last Updated on STN: 9 Jul 2003

L4 ANSWER 2 OF 22 USPATFULL on STN
AN 2003:318218 USPATFULL
TI Methods and reagents for the treatment of multiple sclerosis
IN ***Stratton, Charles W.*** , Nashville, TN, UNITED STATES
Mitchell, William M., Nashville, TN, UNITED STATES
Sriram, Subramaniam, Nashville, TN, UNITED STATES
PI US 2003223959 A1 20031204
AI US 2003-419034 A1 20030417 (10)

RLI Continuation of Ser. No. US 2000-528348, filed on 17 Mar 2000, PENDING
Continuation-in-part of Ser. No. US 1998-73661, filed on 6 May 1998, GRANTED, Pat. No. US 6579854 Continuation-in-part of Ser. No. US 1998-25174, filed on 18 Feb 1998, GRANTED, Pat. No. US 6562582 Continuation-in-part of Ser. No. US 1997-911593, filed on 14 Aug 1997, ABANDONED
PRAI US 1999-125598P 19990319 (60)
US 2000-176662P 20000118 (60)
US 2000-176940P 20000118 (60)
US 2000-176784P 20000118 (60)
DT Utility
FS APPLICATION
LN.CNT 2445
INCL INCLM: 424/085.400
INCLS: 514/028.000; 514/291.000; 514/252.130; 514/192.000; 514/253.080; 514/312.000; 514/152.000; 514/602.000
NCL NCLM: 424/085.400
NCLS: 514/028.000; 514/291.000; 514/252.130; 514/192.000; 514/253.080; 514/312.000; 514/152.000; 514/602.000
IC [7]
ICM: A61K038-21
ICS: A61K031-7048; A61K031-496; A61K031-65; A61K031-18; A61K031-4706
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L4 ANSWER 3 OF 22 USPATFULL on STN
AN 2003:277157 USPATFULL
TI Diagnosis and management of infection caused by ***chlamydia***
IN Mitchell, William M., Nashville, TN, UNITED STATES
Stratton, Charles W. , Nashville, TN, UNITED STATES
PI US 2003195184 A1 20031016
AI US 2002-101279 A1 20020319 (10)
RLI Continuation of Ser. No. US 1998-73661, filed on 6 May 1998, GRANTED, Pat. No. US 6579854 Continuation-in-part of Ser. No. US 1998-25521, filed on 18 Feb 1998, ABANDONED Continuation-in-part of Ser. No. US 1997-911593, filed on 14 Aug 1997, ABANDONED Continuation-in-part of Ser. No. US 1998-73661, filed on 6 May 1998, GRANTED, Pat. No. US 6579854 Continuation-in-part of Ser. No. US 1998-25176, filed on 18 Feb 1998, GRANTED, Pat. No. US 6258532 Continuation-in-part of Ser. No. US 1997-911593, filed on 14 Aug 1997, ABANDONED
PRAI US 1997-45739P 19970506 (60)
US 1997-45779P 19970506 (60)
US 1997-45780P 19970506 (60)
US 1997-45784P 19970506 (60)
US 1997-45787P 19970506 (60)
US 1997-45689P 19970506 (60)
DT Utility
FS APPLICATION
LN.CNT 4849
INCL INCLM: 514/192.000
INCLS: 514/210.090; 514/398.000; 514/471.000; 514/562.000; 514/665.000; 514/574.000
NCL NCLM: 514/192.000
NCLS: 514/210.090; 514/398.000; 514/471.000; 514/562.000; 514/665.000; 514/574.000
IC [7]

ICM: A61K031-407
ICS: A61K031-397; A61K031-198; A61K031-4168; A61K031-19; A61K031-13
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L4 ANSWER 4 OF 22 USPATFULL on STN
AN 2003:250524 USPATFULL
TI Identification of antigenic peptide sequences
IN Mitchell, William M., Nashville, TN, UNITED STATES
Stratton, Charles W. , Nashville, TN, UNITED STATES
PI US 2003175310 A1 20030918
AI US 2001-20269 A1 20011214 (10)
RLI Continuation of Ser. No. US 1998-25596, filed on 18 Feb 1998, GRANTED, Pat. No. US 6340463 Continuation-in-part of Ser. No. US 1997-911593, filed on 14 Aug 1997, ABANDONED
PRAI US 1996-23921P 19960814 (60)
DT Utility
FS APPLICATION
LN.CNT 1728
INCL INCLM: 424/263.100
INCLS: 530/350.000; 530/388.400; 536/023.700
NCL NCLM: 424/263.100
NCLS: 530/350.000; 530/388.400; 536/023.700
IC [7]
ICM: A61K039-118
ICS: C07H021-04; C07K014-295; C07K016-12
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L4 ANSWER 5 OF 22 USPATFULL on STN
AN 2003:244934 USPATFULL
TI Diagnosis and management of infection caused by ***Chlamydia***
IN Mitchell, William M., Nashville, TN, UNITED STATES
Stratton, Charles W. , Nashville, TN, UNITED STATES
PI US 2003171348 A1 20030911
US 6664239 B2 20031216
AI US 2002-100785 A1 20020319 (10)
RLI Continuation of Ser. No. US 1998-73661, filed on 6 May 1998, PENDING Continuation-in-part of Ser. No. US 1998-25521, filed on 18 Feb 1998, ABANDONED Continuation-in-part of Ser. No. US 1997-911593, filed on 14 Aug 1997, ABANDONED
PRAI US 1997-45739P 19970506 (60)
US 1997-45779P 19970506 (60)
US 1997-45780P 19970506 (60)
US 1997-45784P 19970506 (60)
US 1997-45787P 19970506 (60)
US 1997-45689P 19970506 (60)
DT Utility
FS APPLICATION
LN.CNT 4871
INCL INCLM: 514/192.000
INCLS: 514/210.090; 514/398.000; 514/471.000; 514/562.000; 514/574.000; 514/665.000
NCL NCLM: 514/029.000
NCLS: 514/031.000; 514/152.000; 514/179.000; 514/192.000; 514/199.000; 514/311.000; 514/312.000; 514/601.000
IC [7]

ICM: A61K031-397
ICS: A61K031-407; A61K031-4168; A61K031-198; A61K031-19; A61K031-13
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L4 ANSWER 6 OF 22 MEDLINE on STN
AN 2003545283 IN-PROCESS
DN PubMed ID: 14623021
TI Association of ***Chlamydia*** pneumoniae with central nervous system disease.
AU ***Stratton Charles W*** ; Sriram Subramaniam
CS Department of Pathology, Vanderbilt University Medical Center, Nashville, TN, USA.
SO Microbes and infection / Institut Pasteur, (2003 Nov) 5 (13) 1249-53.
Journal code: 100883508. ISSN: 1286-4579.
CY France
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS IN-PROCESS; NONINDEXED; Priority Journals
ED Entered STN: 20031120
Last Updated on STN: 20031219

L4 ANSWER 7 OF 22 MEDLINE on STN
AN 2003261989 MEDLINE
DN 22672019 PubMed ID: 12789196
TI Culture and immunohistochemical evidence of ***Chlamydia*** pneumoniae infection in ulcerative pyoderma gangrenosum.
AU Sams Hunter H; Mitchell William M; ***Stratton Charles W*** ; King Lloyd E Jr
CS Department of Medicine, Vanderbilt University Medical Center, Nashville, Tennesses, USA.
SO JOURNAL OF THE AMERICAN ACADEMY OF DERMATOLOGY, (2003 Jun) 48 (6) 966-9.
Journal code: 7907132. ISSN: 0190-9622.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 200307
ED Entered STN: 20030606
Last Updated on STN: 20030715
Entered Medline: 20030714

L4 ANSWER 8 OF 22 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
DUPLICATE 2
AN 2003:114670 BIOSIS
DN PREV200300114670
TI Dead bugs don't mutate: Susceptibility issues in the emergence of bacterial resistance.
AU ***Stratton, Charles W.*** [Reprint Author]
CS Clinical Microbiology Laboratory, The Vanderbilt Clinic, 21st and Edgehill, Room 4525-TVC, Nashville, TN, 37232, USA
charles.stratton@mcmail.vanderbilt.edu
SO Emerging Infectious Diseases, (January 2003) Vol. 9, No. 1, pp. 10-16.
print.
ISSN: 1080-6040.
DT Article

LA English
ED Entered STN: 26 Feb 2003
Last Updated on STN: 26 Feb 2003

L4 ANSWER 9 OF 22 CAPLUS COPYRIGHT 2004 ACS on STN DUPLICATE 3
AN 2002:65982 CAPLUS
DN 136:133602
TI Identification of antigenic peptide sequences
IN Mitchell, William M.; ***Stratton, Charles W.***
PA Vanderbilt University, USA
SO U.S., 40 pp., Cont.-in-part of U.S. Ser. No. 911,593, abandoned.
CODEN: USXXAM
DT Patent
LA English
FAN.CNT 6

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI US 6340463	B1	20020122	US 1998-25596	19980218
US 2003175310	A1	20030918	US 2001-20269	20011214

PRAI US 1996-23921P P 19960814
US 1997-911593 B2 19970814
US 1998-25596 A1 19980218
RE.CNT 7 THERE ARE 7 CITED REFERENCES AVAILABLE FOR THIS RECORD
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L4 ANSWER 10 OF 22 USPATFULL on STN
AN 2002:16922 USPATFULL
TI ***CHLAMYDIA*** -FREE CELL LINES AND ANIMALS
IN MITCHELL, WILLIAM M., NASHVILLE, TN, UNITED STATES
STRATTON, CHARLES W. , NASHVILLE, TN, UNITED STATES
PI US 2002009802 A1 20020124
US 6562582 B2 20030513
AI US 1998-25174 A1 19980218 (9)
RLI Continuation of Ser. No. US 1997-911593, filed on 14 Aug 1997, ABANDONED
DT Utility
FS APPLICATION
LN.CNT 676
INCL INCLM: 435/325.000
INCLS: 435/031.000; 435/384.000
NCL NCLM: 435/032.000
NCLS: 424/009.100; 424/405.000; 435/031.000; 435/325.000; 435/366.000;
800/014.000
IC [7]
ICM: C12N005-00
ICS: C12N005-02; C12Q001-22
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L4 ANSWER 11 OF 22 CAPLUS COPYRIGHT 2004 ACS on STN DUPLICATE 4
AN 2001:397834 CAPLUS
DN 135:2559
TI Methods for in vitro susceptibility testing of ***Chlamydia***
IN ***Stratton, Charles W.*** ; Mitchell, William M.
PA USA
SO U.S. Pat. Appl. Publ., 9 pp., Cont.-in-part of U.S. Ser. No. 911,593,
abandoned.

CODEN: USXXCO
DT Patent
LA English
FAN.CNT 6

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI US 2001002421	A1	20010531	US 1998-25176	19980218
US 6258532	B1	20010710		
WO 9850074	A2	19981112	WO 1998-US9237	19980506
WO 9850074	A3	19990819		

W: AL, AM, AT, AU, AZ, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, GW, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM

RW: GH, GM, KE, LS, MW, SD, SZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG

AU 9872899	A1	19981127	AU 1998-72899	19980506
AU 746381	B2	20020418		
EP 981372	A2	20000301	EP 1998-920292	19980506

R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, FI

JP 2002512622	T2	20020423	JP 1998-548440	19980506
US 6579854	B1	20030617	US 1998-73661	19980506
US 2003195184	A1	20031016	US 2002-101279	20020319

PRAI US 1997-911593 B2 19970814
US 1996-23921P P 19960814
US 1997-45689P P 19970506
US 1997-45739P P 19970506
US 1997-45779P P 19970506
US 1997-45780P P 19970506
US 1997-45784P P 19970506
US 1997-45787P P 19970506
US 1998-25174 A 19980218
US 1998-25176 A2 19980218
US 1998-25521 A2 19980218
US 1998-73661 A1 19980506
WO 1998-US9237 W 19980506

L4 ANSWER 12 OF 22 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
AN 2001:387345 BIOSIS
DN PREV200100387345
TI Methods for in vitro susceptibility testing of ***chlamydia*** .
AU ***Stratton, Charles W.*** [Inventor, Reprint author]; Mitchell, William M. [Inventor]
CS Nashville, TN, USA
ASSIGNEE: Vanderbilt University
PI US 6258532 July 10, 2001
SO Official Gazette of the United States Patent and Trademark Office Patents, (July 10, 2001) Vol. 1248, No. 2. e-file.
CODEN: OGUPE7. ISSN: 0098-1133.
DT Patent
LA English
ED Entered STN: 15 Aug 2001

Last Updated on STN: 19 Feb 2002

L4 ANSWER 13 OF 22 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
AN 2001:575533 BIOSIS
DN PREV200100575533
TI Get a handle on resistance before it gets a handle on you: The PROTEKT US surveillance study.
AU ***Stratton, Charles W.*** [Reprint author]
CS Department of Medicine, Vanderbilt University Medical Center, 21st and Edgehill, Nashville, TN, 37232, USA
SO Southern Medical Journal, (September, 2001) Vol. 94, No. 9, pp. 891-892. print.
CODEN: SMJOAV. ISSN: 0038-4348.
DT Article
LA English
ED Entered STN: 12 Dec 2001
Last Updated on STN: 25 Feb 2002

L4 ANSWER 14 OF 22 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
DUPLICATE 5
AN 2001:210808 BIOSIS
DN PREV200100210808
TI Regulation by IFN-beta of inducible nitric oxide synthase and interleukin-12/p40 in murine macrophages cultured in the presence of ***Chlamydia*** pneumoniae antigens.
AU Yao, Song-Yi; Ljunggren-Rose, Asa; ***Stratton, Charles W.*** ; Mitchell, William M.; Sriram, Subramaniam [Reprint author]
CS Multiple Sclerosis Research Laboratory, Vanderbilt Stallworth Rehabilitation Hospital, 2201 Capers Avenue, 1222H, Nashville, TN, 37212, USA
srirams@ctrvax.vanderbilt.edu
SO Journal of Interferon and Cytokine Research, (March, 2001) Vol. 21, No. 3, pp. 137-146. print.
ISSN: 1079-9907.
DT Article
LA English
ED Entered STN: 2 May 2001
Last Updated on STN: 18 Feb 2002

L4 ANSWER 15 OF 22 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
AN 2001:362824 BIOSIS
DN PREV200100362824
TI Potential role of ***Chlamydia*** pneumoniae in the pathogenesis of interstitial cystitis.
AU Alberts, Gregory L. [Reprint author]; ***Stratton, Charles W.*** [Reprint author]; Mitchell, William M. [Reprint author]; Franke, Jenny J. [Reprint author]
CS Nashville, TN, USA
SO Journal of Urology, (May, 2001) Vol. 165, No. 5 Supplement, pp. 68. print.
Meeting Info.: Annual Meeting of the American Urological Association, Inc. Anaheim, California, USA. June 02-07, 2001. American Urological Association, Inc.
CODEN: JOURAA. ISSN: 0022-5347.
DT Conference; (Meeting)
Conference; Abstract; (Meeting Abstract)

LA English
ED Entered STN: 2 Aug 2001
Last Updated on STN: 19 Feb 2002

L4 ANSWER 16 OF 22 CAPLUS COPYRIGHT 2004 ACS on STN
AN 2000:688466 CAPLUS
DN 133:249334
TI Methods and reagents for the diagnosis and treatment of multiple sclerosis caused by ***Chlamydia***
IN ***Stratton, Charles W.*** ; Mitchell, William M.; Yao, Song-yi; Bannan, Jason D.; Ljunggren-Rose, Asa; Sriram, Subramaniam
PA Vanderbilt University, USA
SO PCT Int. Appl., 102 pp.
CODEN: PIXXD2
DT Patent
LA English
FAN.CNT 6

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI WO 2000057187	A2	20000928	WO 2000-US7226	20000317
WO 2000057187	A3	20010419		
W: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW: GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG				
EP 1166117	A2	20020102	EP 2000-916513	20000317
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO				

PRAI US 1999-125598P P 19990319
US 2000-176662P P 20000118
US 2000-176784P P 20000118
US 2000-176940P P 20000118
US 2000-528348 A 20000317
WO 2000-US7226 W 20000317

L4 ANSWER 17 OF 22 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
AN 2000:124996 BIOSIS
DN PREV200000124996
TI Pyoderma gangrenosum and ***Chlamydia*** pneumoniae infection in a diabetic man: Pathogenic role or coincidence?.
AU Vannucci, Stephen A.; Mitchell, William M.; ***Stratton, Charles W.*** ; King, Lloyd E., Jr. [Reprint author]
CS Division of Dermatology, Vanderbilt Clinic, Vanderbilt University, Suite 3900, Nashville, TN, 37232-5227, USA
SO Journal of the American Academy of Dermatology, (Feb., 2000) Vol. 42, No. 2 Part 1, pp. 295-297. print.
ISSN: 0190-9622.
DT Article
LA English
ED Entered STN: 5 Apr 2000

Last Updated on STN: 3 Jan 2002

L4 ANSWER 18 OF 22 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
AN 2000:221691 BIOSIS
DN PREV200000221691
TI Induction of IL-12/p40 and NO- by C. pneumoniae antigens in murine macrophages is regulated by beta interferon.
AU Sriram, Subramaniam [Reprint author]; Yao, Song-Yi [Reprint author]; Ljunggren-Rose, O. [Reprint author]; ***Stratton, Charles W.*** [Reprint author]; Mitchell, William M. [Reprint author]
CS Nashville, TN, USA
SO Neurology, (April 11, 2000) Vol. 54, No. 7 Supp. 3, pp. A166. print.
Meeting Info.: 52nd Annual Meeting of the American Academy of Neurology. San Diego, CA, USA. April 29-May 06, 2000. American Academy of Neurology.
CODEN: NEURAI. ISSN: 0028-3878.
DT Conference; (Meeting)
Conference; Abstract; (Meeting Abstract)
LA English
ED Entered STN: 31 May 2000
Last Updated on STN: 5 Jan 2002

L4 ANSWER 19 OF 22 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
AN 1999:160215 BIOSIS
DN PREV199900160215
TI ***Chlamydia*** pneumoniae in patients with interstitial cystitis.
AU Franke, Jenny J.; ***Stratton, Charles W.*** ; Mitchell, William M.
CS Nashville, TN, USA
SO Journal of Urology, (April, 1999) Vol. 161, No. 4 SUPPL., pp. 29. print.
Meeting Info.: 94th Annual Meeting of the American Urological Association, Inc. Dallas, Texas, USA. May 1-6, 1999. American Urological Association.
CODEN: JOURAA. ISSN: 0022-5347.
DT Conference; (Meeting)
Conference; Abstract; (Meeting Abstract)
LA English
ED Entered STN: 16 Apr 1999
Last Updated on STN: 16 Apr 1999

L4 ANSWER 20 OF 22 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
DUPLICATE 6
AN 1999:347842 BIOSIS
DN PREV199900347842
TI ***Chlamydia*** pneumoniae infection of the central nervous system in multiple sclerosis.
AU Sriram, Subramaniam [Reprint author]; ***Stratton, Charles W.*** ; Yao, Song-yi; Tharp, Anthony; Ding, Lingmei; Bannan, Jason D.; Mitchell, William M.
CS Multiple Sclerosis Research Laboratory, 1222H Vanderbilt Stallworth Rehabilitation Hospital, 2201 Capers Avenue, Nashville, TN, 37212, USA
SO Annals of Neurology, (July, 1999) Vol. 46, No. 1, pp. 6-14. print.
CODEN: ANNED3. ISSN: 0364-5134.
DT Article
LA English
ED Entered STN: 24 Aug 1999
Last Updated on STN: 24 Aug 1999

L4 ANSWER 21 OF 22 CAPLUS COPYRIGHT 2004 ACS on STN
AN 1998:752291 CAPLUS
DN 130:10609
TI Diagnosis and management of infection caused by ***Chlamydia***
IN Mitchell, William M.; ***Stratton, Charles W.***
PA Vanderbilt University, USA
SO PCT Int. Appl., 139 pp.
CODEN: PIXXD2
DT Patent
LA English
FAN.CNT 6

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI WO 9850074	A2	19981112	WO 1998-US9237	19980506
WO 9850074	A3	19990819		
W: AL, AM, AT, AU, AZ, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, GW, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW: GH, GM, KE, LS, MW, SD, SZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG				
US 2001002421	A1	20010531	US 1998-25176	19980218
US 6258532	B1	20010710		
AU 9872899	A1	19981127	AU 1998-72899	19980506
AU 746381	B2	20020418		
EP 981372	A2	20000301	EP 1998-920292	19980506
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, FI				
ZA 9803798	A	20000307	ZA 1998-3798	19980506
JP 2002512622	T2	20020423	JP 1998-548440	19980506
PRAI US 1997-45689P	P	19970506		
US 1997-45739P	P	19970506		
US 1997-45779P	P	19970506		
US 1997-45780P	P	19970506		
US 1997-45784P	P	19970506		
US 1997-45787P	P	19970506		
US 1997-911593	A	19970814		
US 1998-25176	A2	19980218		
US 1998-25521	A2	19980218		
US 1998-25174	A	19980218		
WO 1998-US9237	W	19980506		

L4 ANSWER 22 OF 22 CAPLUS COPYRIGHT 2004 ACS on STN
AN 1998:124043 CAPLUS
DN 128:201045
TI Compositions of antichlamydial agents for the diagnosis and management of infection caused by ***chlamydia***
IN Mitchell, William M.; ***Stratton, Charles W.***
PA Vanderbilt University, USA; Mitchell, William M.; Stratton, Charles W.
SO PCT Int. Appl., 83 pp.
CODEN: PIXXD2
DT Patent
LA English

FAN.CNT 6

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI WO 9806435	A2	19980219	WO 1997-US14402	19970814
WO 9806435	A3	19980409		

W: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM

RW: GH, KE, LS, MW, SD, SZ, UG, ZW, AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG

AU 9741516	A1	19980306	AU 1997-41516	19970814
PRAI US 1996-23921P	P	19960814		
WO 1997-US14402	W	19970814		

=>

=> s chlamydia and (trachomatis or pneumoni?)
L5 54802 CHLAMYDIA AND (TRACHOMATIS OR PNEUMONI?)

=> s l5 and (cys ile gly leu ala gly thr asp phe ala asn gln arg pro)
L6 4 L5 AND (CYS ILE GLY LEU ALA GLY THR ASP PHE ALA ASN GLN ARG PRO)

=> dup rem l6
PROCESSING COMPLETED FOR L6
L7 4 DUP REM L6 (0 DUPLICATES REMOVED)

=> d bib ab kwic 1-
YOU HAVE REQUESTED DATA FROM 4 ANSWERS - CONTINUE? Y/(N):y

L7 ANSWER 1 OF 4 USPATFULL on STN
AN 2003:277157 USPATFULL
TI Diagnosis and management of infection caused by ***chlamydia***
IN Mitchell, William M., Nashville, TN, UNITED STATES
Stratton, Charles W., Nashville, TN, UNITED STATES
PI US 2003195184 A1 20031016
AI US 2002-101279 A1 20020319 (10)
RLI Continuation of Ser. No. US 1998-73661, filed on 6 May 1998, GRANTED, Pat. No. US 6579854 Continuation-in-part of Ser. No. US 1998-25521, filed on 18 Feb 1998, ABANDONED Continuation-in-part of Ser. No. US 1997-911593, filed on 14 Aug 1997, ABANDONED Continuation-in-part of Ser. No. US 1998-73661, filed on 6 May 1998, GRANTED, Pat. No. US 6579854 Continuation-in-part of Ser. No. US 1998-25176, filed on 18 Feb 1998, GRANTED, Pat. No. US 6258532 Continuation-in-part of Ser. No. US 1997-911593, filed on 14 Aug 1997, ABANDONED
PRAI US 1997-45739P 19970506 (60)
US 1997-45779P 19970506 (60)
US 1997-45780P 19970506 (60)
US 1997-45784P 19970506 (60)
US 1997-45787P 19970506 (60)
US 1997-45689P 19970506 (60)

DT Utility
FS APPLICATION
LREP CLARK & ELBING LLP, 101 FEDERAL STREET, BOSTON, MA, 02110
CLMN Number of Claims: 36
ECL Exemplary Claim: 1
DRWN 4 Drawing Page(s)
LN.CNT 4849
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The present invention provides a unique approach for the diagnosis and management of infections by ***Chlamydia*** species, particularly C. ***pneumoniae*** . The invention is based, in part, upon the discovery that a combination of agents directed toward the various stages of the chlamydial life cycle is effective in substantially reducing infection. Products comprising combination of antichlamydial agents, novel compositions and pharmaceutical packs are also described.
TI Diagnosis and management of infection caused by ***chlamydia***
AB The present invention provides a unique approach for the diagnosis and management of infections by ***Chlamydia*** species, particularly C. ***pneumoniae*** . The invention is based, in part, upon the discovery that a combination of agents directed toward the various stages of. .

SUMM . . . members of the genus Chlamydiae induces a significant inflammatory response at the cellular level. For example, genital lesions produced by ***Chlamydia*** ***trachomatis*** frequently elicit a vigorous influx of lymphocytes, macrophages, and plasma cells, suggesting the development of humoral and cellular immunity. Yet, clinically, the initial infection is frequently varied in symptomatology and may even be asymptomatic. Once fully established, the ***Chlamydia*** are difficult to eradicate, with frequent relapse following antibiotic therapy. Evidence also indicates that the ***Chlamydia*** may become dormant and are then shed in quantities too few to reliably detect by culture.
SUMM [0005] ***Chlamydia*** ***pneumoniae*** (hereinafter "C. ***pneumoniae*** ") is the most recent addition to the genus Chlamydiae and is isolated from humans and currently is recognized as causing approximately 10 percent of community acquired cases of ***pneumonia*** (Grayston et al., J. Inf. Dis. 161:618-625 (1990)). This newly recognized pathogen commonly infects the upper and lower respiratory tract and is now recognized as ubiquitous in humans. C. ***pneumoniae*** is well-accepted as a human pathogen that may be difficult to eradicate by standard antibiotic therapy (Hammerschlag et al., Clin. Infect. Dis. 14:178-182 (1992)). C. ***pneumoniae*** is known to persist as a silent or mildly symptomatic pathogen, resulting in a chronic, persistent infection (J. Schacter, In: Baun A L, e.g. Microbiology of ***Chlamydia*** , Boca Raton, Fla., CRC Press, 1988, pp. 153-165).
SUMM [0006] The current therapy for suspected/confirmed C. ***pneumoniae*** infection is with a short course (e.g., 2-3 weeks) of a single antibiotic. C. ***pneumoniae*** is susceptible in vitro to tetracyline, erythromycin, clarithromycin, and fluoroquinolones such as ofloxacin and sparfloxacin (Kuo et al., Antimicrob Agents. . . Agents Chemother 38:1873-1878 (1994); M. R. Hammerschlag, Infect. Med. pp. 64-71 (1994)). Despite this demonstration of in vitro susceptibility, C. ***pneumoniae*** infections may relapse following antibiotic therapy with these agents. In vitro studies on the persistence of Chlamydiae

despite specific and. . .

SUMM . . . diagnosis of pathogenic infection as well as therapeutic approaches to manage the infection. Due to the highly infective nature of ***Chlamydia*** EBs and their ability to reinfect cells, there is also a need for antichlamydial therapy which totally eradicates this pathogen,. . .

SUMM [0008] The present invention provides a unique approach for the diagnosis and management of infection by ***Chlamydia*** species, particularly C. ***pneumoniae*** . The invention is based upon the discovery that a combination of agents directed toward many of the various stages of. . . ultimately prevent reinfection/reactivation of the pathogen. Accordingly, one embodiment of the invention pertains to methods of treating infection by a ***Chlamydia*** species, comprising administering to an individual in need thereof a combination of antichlamydial agents, comprising at least two agents, each. . .

SUMM . . . Use of the combination of antichlamydial agents or compositions thereof for the manufacture of a medicament for the management of ***Chlamydia*** infection is also described. In a particular embodiment, the agents can be assembled individually, admixed or instructionally assembled.

SUMM . . . during a course of therapy, the invention provides a means for packaging therapeutic agents, described herein, for the management of ***Chlamydia*** infection. For example, a pack can comprise at least two different agents, each of which is targeted against a different. . .

SUMM . . . the infection status of an individual and/or the progress of therapy in an individual undergoing therapy for infection caused by ***Chlamydia*** . The method comprises quantifying antibody titer or other measure to the pathogen and comparing the measure to antibody measure quantified. . . of the therapy. The invention also pertains to a method for monitoring the course of therapy for treating infection by ***Chlamydia*** , comprising determining presence or absence of ***Chlamydia*** in an infected individual at time intervals during course of therapy. In a particular embodiment, this is determined by PCR. . .

SUMM [0013] Detection of the presence of ***Chlamydia*** in a sample of biological material taken from an individual thought to be infected therewith is important in determining the. . . therapy and the agents to be used. This can be achieved by detecting the presence of DNA encoding MOMP of ***Chlamydia*** or other chlamydial genes in the individual. In one aspect of the invention, diseases associated with ***Chlamydia*** infection, such as inflammatory diseases, autoimmune diseases and diseases in which the individual is immunocompromised, can be treated by managing (i.e., significantly reducing infection or eradicating) the ***Chlamydia*** infection using the novel approach described herein. Both clinical and serological improvements/resolutions in patient status have been demonstrated.

SUMM . . . agent(s) capable of significantly reducing/eliminating chlamydial infection. The method comprises preparing tissue culture from cell lines; inoculating these cells with ***Chlamydia*** in the absence of cycloheximide; allowing the ***Chlamydia*** to infect these cells for several days; adding agent(s) to be tested, which agent(s) is/are replaced as needed for the. . . suitable nucleotide amplification assay, such as PCR. Preferably the presence or absence of signal for amplified DNA encoding MOMP of ***Chlamydia*** or other

chlamydial protein is determined. Absence of a signal indicates a reduction in the degree of infection below that. . . are particularly useful as a drug screening tool for assessing the activity of single agents or combinations of agents against ***Chlamydia*** infection.

SUMM [0016] In one embodiment, a suitable nucleotide assay for identifying agents effective against a cryptic form of ***chlamydia*** comprises, in the presence of agent(s) to be tested, is performed by subjecting cultured cells to protease/reducing agent (e.g., dithiotreitol. . . treated solution; exposing DNA to appropriate polymerase, dNTPs and primers for DNA amplification of MOMP or other protein of the ***Chlamydia*** species; and determining the presence or absence of amplified DNA by visualizing the ethidium bromide treated DNA product by gel electrophoresis, for example. In particular embodiments, the ***Chlamydia*** species is C. ***pneumoniae*** and the appropriate primers are CHLMOMPDB2 and CHLMOMPCB2.

SUMM [0017] The invention further relates to a method of identifying cells containing a cryptic form of a ***Chlamydia*** species by a nucleic acid amplification technique (e.g., PCR) comprising subjecting cultured cells to protease digestion; stopping protease activity; exposing. . . heat-stable DNA polymerase, dNTPs and labeled primers (e.g., 3'-biotin labeled, 5'-biotin labeled) for amplification of DNA encoding MOMP of the ***Chlamydia*** species; washing the cells; exposing the cells to a reporter molecule (e.g., strepavidin-conjugated signal enzyme); exposing the cells to an. . .

SUMM [0018] A method of identifying cells containing a cryptic form of ***Chlamydia*** comprises treating cultured cells, thought to be infected with ***Chlamydia*** , with a disulfide reducing agent; subjecting cultured cells to protease digestion; exposing cells to appropriate polymerase, dNTPs and primers for. . . exposing the cells to an appropriate substrate for the reporter enzyme; and determining the presence of a cryptic form of ***Chlamydia*** by visualizing the amplified DNA encoding a chlamydial protein. Preferably the amplification technique is PCR and the primers are CHLMOMPDB2 and CHLMOMPCB2 of ***Chlamydia*** ***pneumoniae*** .

SUMM . . . similar method can be used as an assay for identifying an agent which is effective against a cryptic form of ***Chlamydia*** . Accordingly, the method comprises treating cultured cells grown in the absence of cycloheximide, thought to be infected with ***Chlamydia*** , with a disulfide reducing agent; allowing the ***chlamydia*** to replicate; adding a test agent; subjecting cultured cells to protease digestion; exposing cells to appropriate polymerase, dNTPs and primers. . . exposing the cells to an appropriate substrate for the reporter enzyme; and determining the presence of a cryptic form of ***Chlamydia*** by visualizing the amplified DNA encoding a chlamydial protein, such as MOMP.

SUMM [0021] The present invention pertains to methods for clearing biological material infected with ***Chlamydia*** to produce ***Chlamydia*** -free cell lines and animals, and to methods of maintaining biological material, e.g., cell lines and animals, such that they remain ***Chlamydia*** -free. According to the method, a biological material is cleared from ***Chlamydia*** infection by contacting the biological material with at least two agents but preferably three agents, each of which is targeted against a different phase of the chlamydial life cycle, until the biological material no longer tests positive for ***Chlamydia*** . The agents can be selected from the

group consisting of a) agents targeted against a cryptic phase of the chlamydial. . .

SUMM [0022] Biological material that has been cleared of ***Chlamydia*** infection, according to the methods of this invention, are also described. The biological material can be a continuous cell line such as HeLa-CF, HL-CF, H-292-CF, HuEVEC-CF and McCoy-CF; wherein "CF" is a shorthand annotation for " ***Chlamydia*** -free". Alternatively, the biological material can be an animal, such as a mouse, rabbit or other animal model, which is negative for ***Chlamydia*** .

SUMM [0023] The invention also pertains to methods of maintaining a ***Chlamydia*** -free status in animals and cell lines which have been cleared of ***Chlamydia*** infection by the methods of this invention, or have never been infected, such as their ***Chlamydia*** -free offspring or progeny. Cells or animals can be maintained as ***Chlamydia*** -free by maintaining them on antibiotics and/or treating their nutrients and environment to ensure that they are ***Chlamydia*** -free. Particularly, a source of nutrients to be administered to ***Chlamydia*** -free cells or animals can be treated to inactivate or remove any chlamydial elementary bodies therefrom. This can be accomplished by. . .

SUMM . . . pertains to a diagnostic kit or pack comprising an assembly of materials selected from the group consisting of antibiotics, reagents, ***Chlamydia*** -free cell lines, and combinations thereof, or other materials that would be necessary to perform any of the methods described herein.

SUMM [0025] The invention further relates to a method of detecting viable ***Chlamydia*** in a biological material suspected of being contaminated therewith, comprising culturing ***Chlamydia*** -free cells or animals in the presence of biological material and then determining the presence or absence of viable ***Chlamydia*** in the culture.

SUMM [0026] The invention also pertains to a method for differentiating porphyria caused by ***Chlamydia*** species from porphyria caused by a genetic disorder. The method comprises measuring peripheral red blood cell enzymes and/or performing a. . . more components of the heme pathway, the porphyria is not caused by a genetic disorder and may be caused by ***Chlamydia*** . The invention relates to a method for diagnosing secondary porphyria caused by ***Chlamydia*** in an individual having symptoms associated therewith, comprising determining the presence or amount of obligatory enzymes in heme biosynthesis in red blood cells of the individual and determining the presence of ***Chlamydia*** in the individual. The invention further relates to a method for differentiating secondary porphyria caused by ***Chlamydia*** from that caused by a genetic disorder in an individual, comprising treating infection by ***Chlamydia*** at many stages of its life cycle and then assessing whether porphyrins have been reduced, wherein a decrease in the porphyrin levels is indicative that the porphyria is secondary and caused by ***Chlamydia*** .

SUMM [0027] The subject invention also pertains to a method for treating porphyria caused by ***Chlamydia*** in an individual in need thereof, comprising reducing the levels of active stage, latent stage and elementary bodies of the. . .

SUMM . . . can be automated using a computerized system, for example, to formulate a drug therapy for management of infection caused by ***Chlamydia*** . The method comprises determining targets within the

chlamydial life cycle, for each determined target; identifying agents that are active against. . . combining at least a subset of the identified agents to provide a combination therapy for management of infection caused by ***Chlamydia*** , the agents in said subset individually being active against different targets in the life cycle of ***Chlamydia*** . The targets include identifying phases of the chlamydial life cycle and for each identified life cycle phase, determining at least. . .

DRWD [0031] FIGS. 1A and 1B show a sequence alignment of various ***Chlamydia*** MOMPs.

DRWD [0033] FIG. 3 illustrates the constant and variable domain (VD) of various ***Chlamydia*** species.

DETD . . . or in combination to eliminate or interfere with more than one of the distinct phases of the life cycle of ***Chlamydia*** species. These chlamydial phases include the intracellular metabolizing/replicating phase; the intracellular "cryptic" phases; and the extracellular EB phase. Current concepts. . .

DETD [0037] Diagnostic and therapeutic methods for the management of ***Chlamydia*** infections are described in detail below. For the purposes of this invention, "management of ***Chlamydia*** infection" is defined as a substantial reduction in the presence of all phases/forms of ***Chlamydia*** in the infected host by treating the host in such a way as to minimize the sequellae of the infection. ***Chlamydia*** infections can thus be managed by a unique approach referred to herein as "combination therapy" which is defined for the. . . or manage chlamydial infection. The diagnostic methods and combination therapies described below are generally applicable for infection caused by any ***Chlamydia*** species, including but not limited to C. ***pneumoniae*** , C. ***trachomatis*** , C. psittaci and C. pecorum. Infections in which the causative agent is C. ***pneumoniae*** are emphasized.

DETD [0038] Antichlamydial agents, which have been identified as effective against ***Chlamydia*** by the susceptibility testing methods described herein, can be used singly to affect ***Chlamydia*** in a single stage of its life cycle or as part of a combination therapy to manage ***Chlamydia*** infection. For example, compounds identified as anti-cryptic phase drugs, anti-EB phase drugs, anti-DNA-dependent RNA polymerase drugs and nicotinic acid cogener. . .

DETD [0039] Diagnosis of ***Chlamydia*** Infection

DETD [0040] The invention pertains to methods for diagnosing the presence of ***Chlamydia*** in a biological material, as well as to the use of these methods to evaluate the serological status of an. . .

DETD . . . more immunoglobulins, such as IgG, IgM, IgA and IgE, against antigenic determinants within the full length recombinant MOMPs of various ***Chlamydia*** species. Detection of IgG and/or IgM against antigenic determinants within the full length recombinant MOMP of C. ***pneumoniae*** is preferred. IgA determinations are useful in the analysis of humoral responses to ***Chlamydia*** in secretions from mucosal surfaces (e.g., lung, GI tract, gerontourinary tract, etc.). Similarly, IgE determinations are useful in the analysis of allergic [manifestatins] manifestations of disease. Table 1 below provides the GenBank Accession numbers of various MOMPs for ***Chlamydia*** species.

TABLE 1

Species	Strain	ID	GenBank Accession No.
C. ***trachomatis***	A	CTL/A	M33636
C. ***trachomatis***	A	CTL/A	M58938
			M33535
C. ***trachomatis***	A	CTL/A	J03813
C. ***trachomatis***	B	CTL/B	M33636
C. ***trachomatis***	C	CTL/L	M17343
			M19128
C. ***trachomatis***	D	CTL/D	A27838
C. ***trachomatis***	E	CTL/E	X52557
C. ***trachomatis***	F	CTL/F	X52080
			M30501
C. ***trachomatis***	H	CTL/H	X16007
C. ***trachomatis***	L1	CTL/L1	M36533
C. ***trachomatis***	L2	CTL/L2	M14738
			M19126
C. ***trachomatis***	L3	CTL/L3	X55700
C. ***trachomatis***	Mouse Pneumo	CTL/MP	X60678
C. pecorum	Ovine Polylarthritis	CPC/OP	Z18756
C. psittaci	Strain 6BC	CPS/6B	X56980
C. psittaci	Feline	CPS/F	X61096
C. ***trachomatis***	Da	CTL/DA	X62921
			S45921
C. ***pneumoniae***	TWAR	CPN/HU1	M64064
			M34922
			M64063
C. ***pneumoniae*** (? C. pecorum)	Horse	CPN/EQ2	L04982
C. ***pneumoniae***	TWAR	CPN/MS	not assigned
C. Psittaci	Horse	CPS/EQ1	L04982

DETD . . . with no cross reactivity to other immunoglobulins (Pharmagen; Clone G20-127, Catalog No. 34152D). Peptide-based immunoassays can be developed which are ***Chlamydia*** specific or provide species specificity, but not necessarily strain specificity within a species, using monoclonal or polyclonal antibodies that are. . .

DETD [0044] Recombinant-based immunological assays have been developed to quantitate the presence of immunoglobulins against the ***Chlamydia*** species. Full length recombinant ***Chlamydia*** MOMP can be synthesized using an appropriate expression system, such as in E. coli or Baculovirus. The expressed protein thus. . . for suitable immunological methods, as discussed above. Protein-based immunological techniques can be designed that are species- and strain-specific for various ***Chlamydia*** .

DETD [0045] Diagnosis of chlamydial infection can now be made with an improved IgM/IgG C. ***pneumoniae*** method of quantitation using ELISA techniques, Western blot confirmation of ELISA specificity and the detection of the MOMP gene of C. ***pneumoniae*** in serum using specific amplification primers that allow isolation of the entire gene for analysis of expected strain-specific differences.

DETD . . . (PCR) methodologies which comprise solution PCR and in situ PCR, to detect the presence or absence of unique genes of

Chlamydia . Species-specific assays for detecting ***Chlamydia*** can be designed based upon the primers selected. Examples of suitable PCR amplification primers are illustrated below in Table 2.. . . ATGAAAAAACTCTTGAAATCGGTATTAGTGTTTGCCGCTTTGAGTTCTGC 17

X55700 CTL/L3 ATGAAAAAACTCTTGAAATCGGTATTAGTGTTTGCCG CTTTGAGTTCTGC 18

X60678 CTL/MP ATGAAAAAACTCTTGAAATCGGTATTAGCATTTGCCG TTTTGGGTTCTGC 19

Chlamydial Species	Strain	SEQ ID NO	Terminal Fifty Nucleotides
C. ***pneumoniae***	TWAR	CPNHU1	GTTTAATTAACGAGAGAGCTGCTCACGTATCTGGTCAGTTCAGATTCTAA 20
C. ***pneumoniae***	MS	CPNHU2	GTTTAATTAACGAGAGAGCTGCTCACGTATCTGG TCAGTTCAGATTCTAA 21
C. psittaci	Horse	CPNEQ1	CAACGTTAATCGACGCTGACAAATGGTCAATCA CTGGTGAAGCACGCTTA 22
C. ***pneumoniae***	Horse	CPNEQ2	GTTTAATTAACGAGAGAGCTGCTCACATATCTGGTCAGTTCAGATTCTAA 23
C. psittaci	SBE	CPS/6B	AACGTTAATCGACGCTGACAAATGGTCAATCAC TGGTGAAGCACGCTTAA 24
C. psittaci	Ewe abortion	CPS/AB1	AACGTTAATCGACGCTGACAAATGGTCAATCAC TGGTGAAGCACGCTTAA 25
C. psittaci	Bovine abortion	CPS/AB2	GCTTAATCAATGAAAGAGCCGCTCACATGAATG CTCAATTCAGATTCTAA 26
C. psittaci	Avian	CPS/AV/C	GCTTAATCAATGAAAGAGCTGCTCACATGAATG CTCAATTCAGATTCTAA 27
C. psittaci	Feline	CPS/F	GCTTAATCGACGAAAGAGCTGCTCACATTAATG CTCAATTCAGATTCTAA 28
C. ***trachomatis***	Hu/A	CTL/A	CGCAGTTACAGTTGAGACTCGCTTGATCGATGAGAGAGCAGCTCACGTAA 29
C. ***trachomatis***	Hu/C	CTL/C	

GCTTGATCGATGAGAGAGCAGGTCACGTAAATGCACAATTCCGGTTCTAA
30

C. ***trachomatis*** Hu/Da CTL/DA
GCTTGATCGATGAGAGAGCAGCTCACGTAAATGCACAATTCCGCTTCTAA
31

C. ***trachomatis*** HU/E CTL/E
CGCTTGATCGATGAGAGACTGCTCACGTAAATGCACAATTCCGCTTCTAA
32

C. ***trachomatis*** HU/F CTL/F
GCTTGATCGATGAGAGAGCTGCTCACGTAAATGCACAATTCCGCTTCTAA
33

C. ***trachomatis*** Hu/H CTL/H
GCTTGATCGATGAGAGAGCAGCTCACGTAAATGCACAATTCCGCTTCTAA
34

C. ***trachomatis*** Hu/L1 CTL/L1
GCTTGATCGATGAGAGAGCTGCTCACGTAAATGCACAATTCCGCTTCTAA
35

C. ***trachomatis*** Hu/L2 CTL/L2
GCTTGATCGATGAGAGAGCTGCTCACGTAAATGCACAATTCCGCTTCTAA
36

C. ***trachomatis*** Hu/L3 CTL/L3
GCTTGATCGATGAGAGAGCAGCTCACGTAAATGCACAATTCCGCTTCTAA
37

C. ***trachomatis*** Mouse CTL/MP
GCTTGATCGATGAAAGAGCAGCTCACGTAAATGCTCAGTTCCGTTTCTAA
38

.sup.aSequence from a cerebral spinal fluid of a patient with multiple sclerosis isolated by the inventors. Sequence is identical to TWAR C. ***pneumoniae*** with exception of a C/T mutation at NT 54 and a G/A mutation at NT 126.
.sup.b Terminator condon underlined
DETD [0047]
TABLE 3

Primers for PCR Amplification of Entire MOMP Gene.sup.a

Chlamydia Species	Strain ID	Plus Strand Primer Sequence	SEQ ID NO.
C. ***pneumoniae***	TWAR	CHLMOMP ATGAAAAAAC TCTTAAAGTC GGCGTTATTA	105
	DB2	TCCGCCGC	61.4.degree.

C. ***trachomatis*** L2 CTMOMP ATGAAAAAAC TCTTGAAATC GGTATTAGTG 61.2.degree. 106
L2DB TTTGCCGCTT TGAG
C. psittaci Feline PSOMP ATGAAAAAAC TCTTAAAATC GGCATTATTA 62.1.degree. 107
FPN-D TTTGCCGCTG CGGG
C. psittaci 6BC PSOMP ATGAAAAAAC TCTTGAAATC GGCATTATTG 63.0.degree. 108
SEC-b TTTGCCGCTA CGGG
C. ***trachomatis*** Mouse CTMU ATGAAAAAAC TCTTGAAATC GGTATTAGCA 63.5.degree. 109
MOMP-D TTTGCCGTTT TGGGTTCTGC

Chlamydia Minus Strand Primer
SEQ ID
Species Strain ID Sequence
t.sub.m.sup.b NO.

C. ***pneumoniae*** TWAR CHLMOMP TTAGAATCTG AACTGACCAG ATACGTGAGC 64.4.degree. 110
CB2 AGCTCTCTCG
C. ***trachomatis*** L2 CTMOMP TTAGAAGCGG AATTGTGCAT TTACGTGAGC 61.5.degree. 111
L2CB AGCTC
C. psittaci Feline PSOMP TTAGAATCTG AATTGAGCAT TAATGTGAGC 62.2.degree. 112
FPN_C AGCTCTTTCG TCG
C. psittaci 6BC PSOMP TTAGAATCTG AATTGACCAT TCATGTGAGC 63.4.degree. 113
GBC_C AGCTCTTTCA TTGATTAAGC G
C. ***trachomatis*** Mouse CTMU TTAGAAACGG AACTGAGCAT TTACGTGAGC 63.2.degree. 114
MOMP_C TGCTCTTTCA TC

.sup.aAll primers amplify under identical amplification conditions: 94.degree. C. for 1. . .

DETD . . . clinical management of the chlamydial infection. Serological improvement can be based upon the current serological criteria for eradication of chronic ***Chlamydia*** reported below in Table 4.

TABLE 4

Serological Criteria for Eradication of Chronic ***Chlamydia*** ***pneumoniae*** Infection

IgM	.ltoreq.1:25
IgG	Stable titer 1:100
PCR	Negative

DETD . . . bromide staining and UV light detection. PCR primers can be designed to selectively amplify DNA encoding MOMP of a particular ***Chlamydia*** species, such as the MOMP of C. ***pneumoniae*** , C. pecorum, C. ***trachomatis*** , C. psittaci (See FIG. 1). Primers that are from about 15-mer to about 40-mer can be designed for this purpose.

DETD [0054] Clearing and Maintaining ***Chlamydia*** -Free Organisms

DETD [0055] The present invention provides a unique approach for creating and

maintaining animals and cell lines which are free of ***Chlamydia*** infection. Also described herein are methods for creating nutrients and culture media that are suitable for use with animals and cell lines that have been cleared of ***Chlamydia*** infection.

DETD [0056] Attempts to culture isolates of C. ***pneumoniae*** from blood and cerebrospinal fluid CSF) have resulted in the discovery that the continuous cell lines routinely used to cultivate C. ***pneumoniae*** are cryptically infected with C. ***pneumoniae*** . These include not only in house stocks of HeLa, HL, H-292, HuEVEC and McCoy cells, but also stocks obtained from. . . for HL cells, as well as a commercial supplier (Bartells) of H-292 and McCoy cells for the clinical culture of ***Chlamydia*** . The presence of a cryptic form of C. ***pneumoniae*** in these cells has been repeatedly demonstrated by solution PCR amplifying the MOMP. In situ PCR in HeLa cells against. . . be present in 100% of cells. Nevertheless, fluoro-scenated mAb to LPS in McCoy cells does not yield any indication of ***Chlamydia*** (i.e., reactive against all ***Chlamydia***) while fluoroscenated mAb to C. ***pneumoniae*** MOMP yields a generalized fluorescence throughout the cytoplasm that can be confused with non-specific autofluorescence. Infection with ***Chlamydia*** ***trachomatis*** (Bartells supply) yields the typical inclusion body staining with the LPS mAb (i.e., cross reactive with all species of ***Chlamydia***) with no change in cytoplasmic signal with anti-MOMP mAb against C. ***pneumoniae*** . These findings (solution PCR, in situ PCR, mAb reactivity) were interpreted as consistent with a cryptic (non-replicating) infection by C. ***pneumoniae*** of cells commonly used to culture the organism. Further, virtually all untreated rabbits and mice tested to date have PCR signals for the C. ***pneumoniae*** MOMP gene.

DETD [0057] This creates a currently unrecognized problem of major significance for those clinical labs providing C. ***pneumoniae*** culture services as well as investigators who now do not know whether their results in animals or in cell culture. . . by cryptic, chlamydial contamination. Clinical and research laboratories currently have no way to determine whether an organism is, in fact, ***Chlamydia*** -free.

DETD [0058] This invention pertains to a method for clearing cells and animals of C. ***pneumoniae*** and keeping them clear. Clearing them entails contacting the infected organism with agents used singly or in combination to eliminate or interfere with more than one of the distinct phases of the life cycle of ***Chlamydia*** species. Keeping them clear entails either maintaining them on antibiotics and/or treating their nutrients and environment to ensure they are ***Chlamydia*** -free. In a preferred embodiment, maintenance conditions comprise a combination of isoniazid (INH) (1 .mu.g/ml), metronidazole (1 .mu.g/ml), and dithiothreitol (10. . .

DETD [0059] These techniques have now made it possible to create a variety of ***Chlamydia*** -free (CF) organisms, including continuous cell lines called HeLa-CF, HL-CF, H-292-CF, HuEVEC-CF, McCoy-CF, African green monkey and other cell lines that. . .

DETD [0060] Because ***Chlamydia*** is highly infectious, organisms which have been cleared of extracellular, replicating and cryptic infections must be protected from exposure to. . . have discovered that many of the nutrients and other materials used to maintain continuous cell lines are contaminated with viable ***Chlamydia*** EBs. For example, every

lot of fetal calf serum has tested positive for the ***Chlamydia*** MOMP gene by PCR. Since extensive digestion is required for isolation of the DNA, we have concluded it is bound in EBs. C. ***pneumoniae*** can also be cultured directly from fetal calf serum. Thus, it is necessary to inactivate EBs in these materials, such as culture media and nutrients, used to maintain the ***Chlamydia*** -free status of the organism. Collectively these materials are referred to herein as "maintenance materials". In one embodiment, nutrients and culture media are subjected to gamma irradiation to inactivate ***Chlamydia*** therein. Preferably, the material should be irradiated for a period of time sufficient to expose the material to at least. . . reducing agent, preferably dithiothreitol (e.g., about 10 .mu.M concentration), before the materials are passed through a filtration system to remove ***Chlamydia*** therefrom.

DETD [0061] In order to insure that research tools, such as cell lines and animals, remain ***Chlamydia*** -free, an assay has been designed to evaluate whether an organism is ***Chlamydia*** -free. The method comprises obtaining a sample of cells or tissue culture; optionally culturing the cells in the presence or absence of cycloheximide; and determining the presence or absence of ***Chlamydia*** nucleic acid by a suitable amplification technique, such as PCR. The absence of nucleic acid amplification signal is indicative that the status of the organism is ***Chlamydia*** -free.

DETD [0062] [Susceptability] Susceptibility Testing for Evaluating Active Agents Against Various Forms of ***Chlamydia***

DETD [0063] This invention pertains to novel approaches for the susceptibility testing of ***Chlamydia*** species that are necessitated by the complex life cycle of the chlamydial pathogen as well as by its diverse, extensive,. . .

DETD . . . to successfully and totally eradicate chronic chlamydial infections. This is because the current susceptibility testing methods measure only replication of ***chlamydia*** and ignores the well-known "cryptic phase" in which intracellular Chlamydiae are not actively replicating. Moreover, it has also been discovered. . .

DETD . . . the invention pertains to methods for evaluating the susceptibility of the distinct phases and stages of the life cycle of ***Chlamydia*** , particularly the cryptic phase to a particular agent(s), since prior techniques have failed, heretofore, to appreciate the need for drugs that can clear infected cells of cryptic ***Chlamydia*** . A preferred drug screening method which accomplished this objective utilizes tissue culture cells which are maintained, in the absence of. . . in order to encourage cryptic infection. Cryptic infection is uncommon in cells used in standard cell culture susceptibility techniques because ***Chlamydia*** in cycloheximide-paralyzed cells need not compete with the host cell for metabolites and hence are encouraged to replicate.

DETD . . . or combination of compounds to be evaluated as an antichlamydial agent for its ability to significantly reduce the presence of ***Chlamydia*** in living cells. For example, a test agent can include, but is not limited to, antibiotics, antimicrobial agents, antiparasitic agents,. . . as PCR) are used to ascertain the presence or absence of signal for chlamydial DNA encoding MOMP or another unique ***Chlamydia*** gene to determine whether the test agent or combination of agents is/are effective in reducing ***Chlamydia*** infection. The loss of signal (i.e., below the

detectable level of the nucleic acid amplification technique) in cells with antibiotic(s) versus its presence in controls is an indication of efficacy of the agent or combination of agents against ***Chlamydia***

.

DETD . . . of this invention can be used to identify an agent or agents which are targeted against any particular species of ***Chlamydia*** and can be used to identify agent(s) targeted against the cryptic form of the pathogen, i.e., is capable of inhibiting. . . embodiment, this is done by performing the susceptibility test while placing the cells under stringent environmental conditions known to induce .***Chlamydia*** to enter a cryptic phase. Agents that are effective against ***Chlamydia*** , as ascertained by the susceptibility testing protocols described herein, can be used as part of a therapy for the management of ***Chlamydia*** infections. Suitable therapeutic protocols are described in detail below, with a particular focus on targeting agents toward specific stages of. . .

DETD [0072] In one embodiment, a suitable nucleic acid assay for identifying agents effective against the cryptic form of ***Chlamydia*** comprises, in the presence of agent(s) to be tested, subjecting cultured cells to reducing agent (e.g., dithiotreitol) and protease digestion. . . . treated solution; exposing DNA to appropriate polymerase, dNTPs and primers for DNA amplification of MOMP or other protein of the ***Chlamydia*** species; and determining the presence or absence of amplified DNA by visualizing the ethidium bromide treated DNA product by gel electrophoresis, for example, or alternatively by Southern Blot. In particular embodiments, the ***Chlamydia*** species is C. ***pneumoniae*** and the appropriate primers are CHLMOMPDB2 and CHLMOMPCB2.

DETD [0073] The invention further relates to a method of identifying cells containing a non-EB cryptic form of a ***Chlamydia*** species by a nucleic acid amplification technique (e.g., PCR) comprising subjecting cultured cells to protease digestion; stopping protease activity; exposing. . . heat-stable DNA polymerase, dNTPs and labeled primers (e.g., 3'-biotin labeled, 5'-biotin labeled) for amplification of DNA encoding MOMP of the ***Chlamydia*** species; washing the cells; exposing the cells to a reporter molecule (e.g., strepavidin-conjugated signal enzyme); exposing the cells to an. . .

DETD [0074] The invention pertains to a method of identifying cells containing a cryptic form of ***Chlamydia*** . The method comprises treating cultured cells, thought to be infected with ***Chlamydia*** , with a disulfide reducing agent; subjecting cultured cells to protease digestion; exposing cells to appropriate polymerase, dNTPs and primers for. . . exposing the cells to an appropriate substrate for the reporter enzyme; and determining the presence of a cryptic form of ***Chlamydia*** by visualizing the amplified DNA encoding a chlamydial protein. Preferably, the amplification technique is PCR and the primers are CHLMOMPDB2 and CHLMOMPCB2 of ***Chlamydia*** ***pneumoniae***

.

DETD . . . similar method can be used as an assay for identifying an agent which is effective against a cryptic form of ***Chlamydia*** . Accordingly, the method comprises treating cultured cells grown in the absence of cycloheximide, thought to be infected with ***Chlamydia*** , with a disulfide reducing agent; allowing the ***Chlamydia*** to replicate; adding a test agent; subjecting cultured cells to protease digestion; exposing cells to appropriate polymerase, dNTPs and primers.

. . enzyme; exposing the cells to an appropriate substrate for the reporter enzyme; and determining the presence of cryptic form of ***Chlamydia*** by visualizing the amplified DNA encoding a chlamydial protein, such as MOMP.

DETD . . . susceptibility test can be used to evaluate the status of a human or animal undergoing therapy for the management of ***Chlamydia*** infection. For example, a biological material is isolated from the human or animal to undergo combination therapy. The biological material is treated such that the ***Chlamydia*** is isolated therefrom. This chlamydial isolate is allowed to infect ***Chlamydia*** free cells. These infected cells are then exposed to the combination of agents being used in the individual undergoing combination. . .

DETD . . . method has revealed, for example, that antimicrobial therapy with the triple agents, INH, metronidazole and penicillamine, can completely eradicate C. ***pneumoniae*** from infected mice in four months. Moreover, following complete eradication of chlamydiae, multiple attempts to reinfect these cured mice via. . .

DETD . . . of determining the presence of cryptic chlamydial infections in an animal or cell culture is to expose the culture to ***chlamydia*** -stimulating compounds. Such compounds include (but are not limited to) cycloheximide, corticosteriods (such as prednisone) and other compounds which are known. . .

DETD [0081] Antichlamydial Therapy Directed Toward the Initial Stage of ***Chlamydia*** Infection

DETD . . . and electron transfer proteins, as well as nitroreductases. Based upon this, it has been discovered that the initial phase of ***Chlamydia*** infection is susceptible to the antimicrobial effects of nitroimidazoles, nitrofurans and other agents directed against anaerobic metabolism in bacteria.

DETD . . . including ribosomes, DNA and RNA. Nitroimidazoles and nitrofurans currently are not considered to possess antimicrobial activity against members of the ***Chlamydia*** species. This lack of antimicrobial activity, however, is due to the fact that conventional susceptibility testing methods only test for effect on the replicating form of ***Chlamydia*** species.

DETD . . . an agent, such that the modification results in an agent having similar or increased, but not significantly decreased, effectiveness against ***Chlamydia*** , compared to the effectiveness of the parent agent from which the analog or derivative is obtained. This comparison can be. . .

DETD [0087] Novel Antichlamydial Therapy Directed Toward the Replicating and Cryptic Stationary Phases of ***Chlamydia*** Infection

DETD [0088] A unique class of antichlamydial agents that is effective against the replicating and cryptic stationary phases of ***Chlamydia*** (and possibly against some other stages of the cryptic phase) have been identified using the susceptibility tests described herein. This. . . [susceptability] susceptibility testing methodologies, it has been discovered that these agents, in combination with other antibiotics, are particularly effective against ***Chlamydia*** . It is believed that the isonicotinic acid congeners target the constitutive production of catalase and peroxidase, which is a characteristic of microorganisms, such as mycobacteria, that infect monocytes and macrophages. ***Chlamydia*** can also successfully infect monocytes and macrophages.

DETD [0089] Using INH to eradicate ***Chlamydia*** from macrophages and monocytes subsequently assists these cells in their role of fighting infection. However, these agents appear to be. . .

DETD . . . and its congeners can be used to clear infection from monocytes and/or macrophages. When monocytes and macrophages are infected by ***Chlamydia*** , they become debilitated and cannot properly or effectively fight infection. It is believed that, if the chlamydial infection, per se,. . . one aspect of the invention provides a specific method for reempowering monocytes or macrophages that have been compromised by a ***Chlamydia*** infection and, in turn, comprise treating the infection in other sites. Such compromised macrophages or monocytes can be activated by. . .

DETD [0091] Therapy Directed Toward Elementary Bodies of ***Chlamydia***

DETD . . . discovered that adverse conditions, such as limited nutrients, antimicrobial agents, and the host immune response, produce a stringent response in ***Chlamydia*** . Such adverse conditions are known to induce stringent responses in other microorganisms (C. W. Stratton, In: Antibiotics in Laboratory Medicine,. . . Fourth Edition. Lorian V (ed) Williams & Wilkins, Baltimore, pp 579-603 (1996)) and not surprisingly induce a stringent response in ***Chlamydia*** . This stringent response in ***Chlamydia*** alters the morphological state of the intracellular microorganism and creates dormant forms, including the intracellular EB, which then can cryptically. . . the extracellular milieu. Thus, it is necessary to utilize a combination of agents directed toward the various life stages of ***Chlamydia*** and, in particular, against the elementary body for successful management of infection.

DETD . . . also believed that [persistance] persistence of chlamydial infections, in part, may be due to the presence of cryptic forms of ***Chlamydia*** within the cells. This cryptic intracellular chlamydial form apparently can be activated by certain host factors such as cortisone (Yang. . . and Immunity, 39:655-658 (1983); and Malinverni et al., The Journal of Infectious Diseases, 172:593-10 594 (1995)). Antichlamydial therapy for chronic ***Chlamydia*** infections must be continued until any intracellular EBs or other intracellular cryptic forms have been activated and extracellular EBs have. . .

DETD . . . their respective hosts by reducing disulfide bonds which maintain the integrity of the outer membrane proteins of the EBs. For ***Chlamydia*** , disruption of the outer membrane proteins of EBs thereby initiates the transition of the EB form to the RB form.. . .

DETD [0098] Currently Recognized Agents Active Against ***Chlamydia*** Replication

DETD . . . they begin to utilize active transcription of chlamydial DNA and translation of the resulting mRNA. As such, these forms of ***Chlamydia*** are susceptible to currently used antimicrobial agents. The antichlamydial effectiveness of these agents can be significantly improved by using them in combination with other agents directed at different stages of ***Chlamydia*** life cycle, as discussed herein.

DETD . . . as well as those which are preferred, are illustrated below in Table 5.

TABLE 5

Agents Effective Against the Replicating Phase of ***Chlamydia***

Drug Class	Examples	Preferred
Quinolones/ Fluoroquinolones	Ofloxacin	Levofloxacin
	Levofloxacin	
	Trovafloxacin	
	Sparfloxacin	
	Norfloxacin	
	Lomefloxacin	
	Cinoxacin	
	Enoxacin	
	Nalidixic Acid	
	Fleroxacin	
	Ciprofloxacin	
Sulfonamides. . .		

DETD [0101] All members of the ***Chlamydia*** species, including C. ***pneumoniae*** , are considered to be inhibited, and some killed, by the use of a single agent selected from currently used antimicrobial agents such as those described above. However, using the new susceptability test, the inventors have found complete eradication of ***Chlamydia*** cannot be achieved by the use of any one of these agents alone because none are efficacious against all phases of the ***Chlamydia*** life cycle and appear to induce a stringent response in ***Chlamydia*** causing the replicating phase to transform into cryptic forms. This results in a persistent infection in vivo or in vitro. . . DNA. Nevertheless, one or more of these currently used agents, or a new agent directed against the replicating phase of ***Chlamydia*** , should be included as one of the chlamydial agents in a combination therapy in order to slow or halt the. . .

DETD . . . attempting to manage or eradicate a systemic infection, it is critical to target multiple phases in the life cycle of ***Chlamydia*** , otherwise viable ***Chlamydia*** in the untargeted phases will remain after therapy and result in continued, chronic infection. This fundamental insight is at the. . .

DETD [0111] 2. Evaluate the relative importance of targeting each particular phase in eradicating reservoirs of ***Chlamydia*** from the host organism. For example, the life-cycle stages listed in step 1 can be prioritized based on the following. . .

DETD . . . reproduction cycle seen in cycloheximide-treated eukaryotic cells is an artifact of an atypical, cell culture environment designed primarily to propagate ***Chlamydia*** .

DETD [0114] c. The transition phases represent only a small portion of ***Chlamydia*** in chronic infections.

DETD [0115] 3. Identify "targets" for each phase of the selected life cycle phases. A target is an attribute of ***Chlamydia*** which is vulnerable during a particular life cycle phase. For example, the disulfide bonds in MOMP are a target during. . .

DETD . . . of the chlamydial life cycle leads to a re-prioritization or even sub-division of the life-cycle phases, new theoretical targets within ***Chlamydia*** are identified, or new drugs are developed which attack currently known or new targets within ***Chlamydia*** . For example, the phases of the life cycle could be further sub-classified based on the type of host cell the. . . Therapy

Rel-
Potentially production
DNA- Ribosomes ative
vulnerable of
dependent involved in Im-
attributes of Disulfide Non-oxidative peroxidases
RNA Folic acid Protein por-
Chlamydia : bonds metabolism and
catalyses Topoisomerases polymerase pathway synthesis
tance

Phase in Chlamydial Life Cycle | Theoretical Targets
EB (Extracellular or Intracellular) | X
8
EB->RB Transition. . .
DETD [0132] An association has been discovered between chronic ***Chlamydia*** infection of body fluids and/or tissues with several disease syndromes of previously unknown etiology in humans which respond to unique. . . neural-mediated hypotension); Pyoderma Gangrenosum (PG), Chronic Fatigue (CF) and Chronic Fatigue Syndrome (CFS). Other diseases are under investigation. Correlation between ***Chlamydia*** infection and these diseases has only recently been established as a result of the diagnostic methodologies and combination therapies described. . .
DETD [0133] Based on this evidence, published evidence of an association between atherosclerosis and ***Chlamydia*** (Grupta et al., Circulation ,96:404-407 (1997)), and an understanding of the impact ***Chlamydia*** infections have on infected cells and the immune systems, the inventors have discovered a connection between ***Chlamydia*** and a broad set of inflammatory, autoimmune, and immune deficiency diseases. Thus, the invention describes methods for diagnosing and/or treating disease associated with ***Chlamydia*** infection, such as autoimmune diseases, inflammatory diseases and diseases that occur in immunocompromised individuals by diagnosing and/or treating the ***Chlamydia*** infection in an individual in need thereof, using any of the assays or therapies described herein. Progress of the treatment can be evaluated serologically, to determine the presence or absence of ***Chlamydia*** using for example the diagnostic methods provided herein, and this value can be compared to serological values taken earlier in. . . alternate compounds should be substituted in order to achieve the lower antibody titers that demonstrate specific [susceptability] susceptibility of the ***Chlamydia*** to the new regimen. A replacement or substitution of one agent with another agent that is effective against the same life stage of ***Chlamydia*** is desirable.
DETD . . . be used for the treatment of acute and chronic immune and autoimmune diseases when patients are demonstrated to have a ***Chlamydia*** load by the diagnostic procedures described herein which diseases include, but are not limited to, chronic hepatitis, systemic lupus erythematosus,. . .
DETD . . . peripheral neuropathy, chronic or recurrent sore throat, laryngitis, tracheobronchitis, chronic vascular headaches (including migraines, cluster headaches and tension headaches) and

pneumonia when demonstrated to be pathogenically related to ***Chlamydia*** infection.

DETD [0137] Treatable disorders when associated with ***Chlamydia*** infection also include, but are not limited to, neurodegenerative diseases, including, but not limited to, demyelinating diseases, such as multiple. . .

DETD . . . the diseases indicated were observed and are reported in Example 5. The data provides evidence to establish that treatment of ***Chlamydia*** infection results in the serological and physical improvement of a disease state in the patient undergoing combination therapy. These observations. . .

DETD [0141] Other Diseases of Unknown Etiology with New Evidence for A ***Chlamydia*** ***Pneumoniae*** Etiology

DETD [0142] Both C. ***trachomatis*** and C. psittaci exhibit a protean disease complex dependent on different serovars. One known basis for this diversity to date is the amino acid sequence of the MOMP. FIG. 1 shows a sequence alignment of various ***Chlamydia*** MOMPs. Note that the size and sequence are relatively homologous except for the four variable regions that are responsible for the serovar (serotype) basis of classification. Further, it has been discovered that C. ***pneumoniae*** infects blood vessel endothelial cells from which EBs are released in the blood stream. In addition, macrophages are known targets for C. ***pneumoniae*** and may serve as reservoirs and provide an additional mechanism of transmission. C. ***pneumoniae*** is thus able to spread throughout the human body, establishing infection in multiple sites and in multiple organ systems. Infected. . .

DETD . . . intended to embrace both humans and animals. Virtually all rabbits and mice tested to date have PCR signals for C. ***pneumoniae*** . They can be used as appropriate animal models for treatment using specific combination antibiotics to improve therapy. (Banks et al.,. . .

DETD [0144] Coupled with these developments are the recently developed rabbit models of coronary artery disease, where rabbits exposed to C. ***pneumoniae*** subsequently develop arterial plaques similar to humans (Fong et al., J. Clin. Microbiol. 35:48-52 (1997)). Most recently, a study at. . . George's Hospital in London found that roughly 3/4 of 213 heart attach victims have significant levels of antibodies to C. ***pneumoniae*** antibody and that those that have such antibodies achieve significantly lower rates of further adverse cardiac events when treated with. . .

DETD . . . also been introduced based on the report that Vitamin C (ascorbic acid) at moderate intracellular concentrations stimulates replication of C. ***trachomatis*** (Wang et al., J. Clin. Micro. 30:2551-2554 (1992)) as well as its potential effect on biofilm charge and infectivity of. . .

DETD [0151] ***Chlamydia*** is a parasite of normal energy production in infected eukaryotic cells. As a result, host cells have insufficient energy available. . . cell mitochondria to attempt to synthesize certain critical enzymes involved in energy production in order to increase energy production. Because ***Chlamydia*** also prevents this synthesis from completing, these enzyme's precursors, called porphyrins, build up in cell and often escape into the. . .

DETD . . . this secondary form of porphyria, a unique approach for the diagnosis and treatment of obligatory and secondary disorders caused by ***Chlamydia*** infections has been developed. The adjunctive therapy

described herein can be used in combination with the appropriate antimicrobial therapy required. . .

DETD . . . Y., Microbiological Reviews, 42:247-306 (1978); McClairty, G., Microbiology, 2:157-164(1994)). The transition of elementary bodies (EBs) to reticulate bodies (RBs) for ***Chlamydia*** species requires the presence of functioning mitochondria in the infected cell as well as the production by the host cell. . .

DETD [0165] B. ***Chlamydia*** and Secondary Porphyria

DETD . . . step in the biosynthesis of heme as it catalyses the oxidative entry of coproporphyrinogen into the mitochondria matrix as protoporphyrin; ***Chlamydia*** interfere with this step by reducing electron transfer in the host cell. When coproporphyrinogen is unable to return to the. . .

DETD [0167] Depletion of host cell energy by the intracellular infection with ***Chlamydia*** species causes additional energy-related complications. As fewer electrons are available to move through the electron transport chain of the host. . .

DETD . . . the classical manifestations of hereditary porphyria. As the chlamydial-infected host cells lyse, as happens in the normal life cycle of ***Chlamydia*** , the intracellular porphyrins are released and result in a secondary porphyria. Moreover, when the chlamydial infection involves hepatic cells, the. . . is a heme-based enzyme. Hence, the biosynthesis of heme in the liver becomes increased. When hepatic cells are infected with ***Chlamydia*** species, the decreased energy in the host cell does not allow heme biosynthesis to go to completion and porphyrins in the liver/entero-hepatic circulation are increased. It also has been noted that any host cell infected with ***Chlamydia*** species has an increased amount of intracellular porphyrins that are released when antimicrobial agents kill the microorganism.

DETD . . . clearly is of paramount importance in dealing with chronic systemic chlamydial infections as are seen with intravascular infections caused by ***Chlamydia*** ***pneumoniae*** .

DETD . . . (Kordac V., Neoplasma, 19:135-139 (1972); Lithner et al., Acta Medica Scandanavia, 215:271-274 (1984)). Of particular interest is that infection with ***Chlamydia*** ***pneumoniae*** has been associated with lung cancer (Cerutti P A., Science, 227:375-381 (1985)).

DETD . . . foregoing discussion of the etiology of porphyria, one aspect of the invention pertains to methods for differentiating porphyria caused by ***Chlamydia*** from that caused by a latent genetic disorder in an individual. The method comprises treating infection by ***Chlamydia*** at all stages of its life cycle, using the therapies described in detail elsewhere in this disclosure, and then assessing. . . symptoms of porphyria (e.g., biochemical, enzymatic or physical manifestation) are indicative that the porphyria is a secondary porphyria caused by ***Chlamydia*** .

DETD . . . is suggestive of a non-genetic porphyria, such as chlamydially induced secondary porphyria. For example, in one embodiment, porphyria caused by ***Chlamydia*** in an individual having symptoms associated therewith can be diagnosed by determining the presence and/or amount of obligatory enzymes in. . .

DETD [0175] As discussed above, some patients having a ***Chlamydia*** -induced porphyria do not have abnormal levels of heme precursors. For those patients it may be appropriate to determine the presence of ***Chlamydia*** as well as porphyrins in the individual. The presence of both the pathogen and porphyrins (e.g., determined by ELISA assay. .

DETD . . . well as Vitamin B12 (cobalamin), which is molecularly similar to these metabolites, in patients with active systemic infection with C. ***pneumoniae*** . The antibodies are primarily IgM; this is similar to the antibody responses to the MOMP of C. ***pneumoniae*** in severely symptomatic patients. Example 8 illustrates titers in symptomatic patients with systemic C. ***pneumoniae*** infections. The presence of antibodies to Vitamin B12 may have functional significance by decreasing the amount of bioavailable Vitamin B12. Thus, a ***Chlamydia*** infection may cause a previously unrecognized secondary Vitamin B12 deficiency. Administration (e.g., intramuscular) of large quantities of Vitamin B12 (1000. . .

DETD [0179] Treatment of ***Chlamydia*** infection may [exaserbate] exacerbate secondary porphyria by increasing the metabolism of cryptic ***Chlamydia*** or by accelerating the death of infected cells with elevated intracellular porphyrin levels.

DETD [0194] To reduce severe porphyric attacks during therapy for chronic ***Chlamydia*** infections, the use of hemodialysis, plasmapheresis, chelating agents and/or intravenous hematin may be needed. Any one of these or a. . .

DETD . . . nutritional formulations including beverages and foods such as nutritional bar, for the management of non-genetic, secondary porphyria caused by a ***Chlamydia*** infection. Alternatively, a combination of vitamins and antioxidants can be co-packed in a pack or kit as described elsewhere herein. . .

DETD . . . to ameliorate conditions/symptoms associated with the disease states described above, when the disease is onset or aggravated by infection by ***Chlamydia*** . The agents of this invention can be administered to animals including, but not limited to, fish, amphibians, reptiles, avians and. . .

DETD . . . thereof. The agents can also be used for the manufacture of a medicament for therapy of a disease associated with ***chlamydia*** infection, such as autoimmune disease, inflammatory disease, immunodeficiency disease.

DETD Polymerase Chain Reaction (PCR) for the Full Length MOMP Gene of C. ***Pneumoniae*** and Other Species of ***Chlamydia*** (Diagnostic)

DETD . . . which on repeated assay without reducing agents, yields a negative PCR signal for the 1.2 kB MOMP gene of C. ***pneumoniae*** . Analysis on agarose gel with ethidium bromide visualization under UV light.

DETD . . . signals using the preferred primers which amplify the full length MOMP gene suggests that mutations in these regions of C. ***pneumoniae*** is rare. Standard conditions for this gene product in a 50-.mu.l volume is 35 cycles with 1 second ramp times. . .

DETD [0225] This procedure identifies individual cells containing RB and cryptic forms of C. ***pneumoniae*** . Cultured cells are adhered to glass slides with formalin, or formalin fixed tissue sections embedded in paraffin are adhered to. . .

DETD [0228] The full length MOMP gene of C. ***pneumoniae*** was directionally cloned into the pET expression plasmid at the NCOI and NOTI restriction sites using primers to introduce these. . .

DETD . . . length expressed recombinant fusion protein or the modified MOMP following endopeptidase cleavage can be used as the antigen for a ***Chlamydia*** species ELISA.

DETD [0232] The recombinant MOMP-based ELISA described above provides a

sensitive method for the quantitation of immunoglobulins against the ***Chlamydia*** genus in serum, plasma, CSF, and other body fluids. In order to provide ELISA assays that are species- and potentially strain-specific for the various ***Chlamydia*** , two regions in the MOMP have been identified which show minimal amino acid sequence homologies and which are predicted by. . . methodology parallels that described above for the recombinant MOMP-based ELISA. In addition, a highly antigenic-domain (FIG. 6) common to all ***Chlamydia*** has been identified and was developed as an alternative genus-specific ELISA for the ***Chlamydia*** . Immunization of rabbits has verified the antigenicity of each peptide to each peptide (Table 9). Monoclonal antibodies have further verified. . . computer analysis of the nucleotide-generated amino acid sequence of each species-specific MOMP.

TABLE 9

Antigenic Responses To Peptides From 4 Species of ***Chlamydia*** Identified By Hydrophilicity And Peptide Movement As Highly Antigenic

Chlamydia Species	Peptide.sup.b	Titer.sup.a Pre	Post
C. ***pneumoniae***	90-105	100	>3200
C. ***trachomatis*** L2	91-106	800	>3200
C. psittaci	92-106	400	>3200
C. ***trachomatis*** (mouse)	89-105	0	>3200
C. ***pneumoniae***	158-171	25	>3200
C. ***trachomatis*** L2	159-175	200	>3200
C. psittaci	160-172	100	>3200
C. ***trachomatis*** (mouse)	158-171	800	>3200
C. ***pneumoniae***	342-354	200	>3200
C. ***trachomatis*** L2	342-354	100	>3200
C. psittaci	ND.sup.c		
C. ***trachomatis*** (mouse)	ND.sup.c		

.sup.aReciprocal titer

.sup.bImmunogenic peptide and ELISA antigen of specific amino acid sequence against the indicated pre-immunization and post-immunization rabbit. .

.

DETD [0233] Table 10 illustrates reciprocal titers of a polyclonal and monoclonal antibody against C. ***trachomatis*** cross-reactive against C. ***pneumoniae*** peptide encompassing amino acids 342-354 and a recombinant full length MOMP from C. ***pneumoniae*** .

TABLE 10

Reciprocal titers of a polyclonal and a monoclonal antibody against C. ***trachomatis*** cross-reactive against C. ***pneumoniae*** peptide encompassing amino acids 342-354 and a recombinant full length MOMP from C. ***pneumonia*** .

Antigen	Titer.sup.a Polyclonal Ab.sup.b	Monoclonal Ab.sup.c

CPN Momp.sup.d	400	0
CPN 90-105.sup.e	50	0
CPN 158-171.sup.f	50	0
CPN 342-354.sup.g	>3200	1600

.sup.aReciprocal titer
.sup.bPolyclonal goat Ab from Chemicon Inc. against MOMP of C. ***trachomatis***
.sup.cMonoclonal Ab (ICN, Inc.) against MOMP of C. ***trachomatis***
.sup.dC. ***pneumoniae*** recombinant MOMP
.sup.eAmino acid peptide 90-105 of C. ***pneumoniae***
.sup.fAmino acid peptide 158-171 of C. ***pneumoniae***
.sup.gAmino acid peptide 342-354 of C. ***pneumoniae***

DETD [0235] C. ***pneumoniae*** EBs were grown in primary human umbilical vein endothelial cells (HuEVEC; early passage), HeLa 199, or a suitable alternative in. . .

DETD [0237] Western blots were prepared by SDS-PAGE of C. ***pneumoniae*** EBs (non-formalin fixed) harvested from infected HuEVEC or HeLa cell lysates, electrophoresed under standard SDS-PAGE conditions, and transferred to nitrocellulose. . .

DETD In Vitro Antimicrobial Susceptibility Testing for C. ***Pneumoniae***

DETD [0240] Tissue culture cells containing cryptic phase C. ***pneumoniae*** (H-292, HeLa, HEL, HuEVEC, McCoy, etc.) are plated at subconfluency in a 96-well microtiter plate (flasks or plates or other. . .

DETD . . . at 1 .mu.g/ml failed to clear HeLa cells in culture of a detectable PCR signal for the MOMP gene of ***Chlamydia*** ***pneumoniae*** . In contrast, triple agents consisting of isoniazid (INH), metronidazole, and penicillamine (1 .mu.g/ml each) resulted in no detectable PCR signal. . .

DETD . . . the methodology described in the section above entitled "Methodology for Selecting Potential Agent Combinations".

TABLE 11

Susceptibility to Antibiotics for
Cryptic C. ***pneumoniae*** Cultured in HeLa Cells.sup.a

Antibiotic	Cone (.mu.g/ml)	PCR.sup.b
Ofloxacin	1	positive
Clarithromycin	1	positive
INH	1	positive
Metronidazole	1	positive

. . .

DETD [0243]

TABLE 12

Susceptibility to Antibiotics by PCR for
Cryptic ***Chlamydia*** ***pneumoniae*** Cultured in HeLa Cells.sup.1
Phase of the Chlamydial Life Cycle

EB (Extracellular	EB->RB Transition	Stationary Phase RB Concentration	PCR	RB->EB PCR. . .

DETD . . . typical responses to combination antibiotic therapy in a

variety of patients with diagnostic evidence of an active infection by C. ***pneumoniae*** . Unlike typical immune responses to infection with infectious agents, most of the included patients have not only detectable IgM titers. . . the IgM titers generally fall, with a rise in IgG titer (as expected). Current methods of detecting antibodies against C. ***pneumoniae*** (Indirect immunofluoresence, MIF) are incapable of accurately identifying high IgM titers against C. ***pneumoniae*** . Moreover, current procedures are genus specific and not species specific as are peptide-based ELISAs.

DETD . . . months with an EDSS = 8.0 (triplegic plus speech and swallowing impairments). A positive CSF PCR and culture for C. ***pneumoniae*** led to treatment with combination antibiotics. The patient improved on all spheres of neurologic function over the following six months.. . . legs. Over 5 months his EDSS score worsened from 7.0 to 8.0. His CSF was positive by PCR for C. ***pneumoniae*** and he was placed on combination antibiotics. Over the next six months he gradually improved in his balance, coordination and. . . to response to corticosteroids on two successive occasions. Five months later, his EDSS score was 7.5. Following a positive C. ***pneumoniae*** PCR he was placed on combination antibiotics. He has gradually gained strength in his lower extremities and five months later. . . progressive MS with recent progressive bulbar symptoms, axtaxia, and paraplegia (EDSS = 8.5). PCR for the MOMP gene of C. ***pneumoniae*** in the CSF was positive. She was placed on combination antibiotics with no further progression of symptoms for the last. . . ulcers improved again.

TW PG Severe PG, initiated after a chemical burn in 1991, but with PCR negative serology for C. ***pneumoniae*** . Patient did not initially respond to combination antibiotic therapy. A positive biopsy culture for C. ***pneumoniae*** resulted in the recent re-institution of combination antibiotics. However, after no improvement, patient went off therapy.

AM IBD Row 5 This. . . the colectomy, the patient experienced neurologic symptoms, fatigue, myalgias. arthralgias, and an acneoform skin rash. Serology was performed for C. ***pneumoniae*** and was positive with an IgM of 1:3200, IgG 1:400 and PCR positive. Therapy with combination antibiotics was initiated.

After. . . resolution
of her proctitis on visual exam.
NM CFS Vanderbilt University initial patient that resulted in our first association of
C. ***pneumoniae*** , initially complained of the insidious onset of debilitating fatigue.
This was associated with a severe cognitive dysfunction that disrupted. . . Infectious Disease Clinic at
Vanderbilt no definitive or presumptive diagnosis could be made. A subsequent
high antibody titer against C. ***pneumoniae*** led to standard anti-chlamydial
antibiotic therapy over a three month period with gradual disappearance of
fatigue and cognition symptoms. On. . . acute anxiety attacks relieved by anti-
porphyrin therapy.
WM CF Row 7 CF following acute stress. Pre-illness serum negative for anti-
Chlamydia ***pneumoniae*** antibodies which peaked six
weeks following stress. Pre-illness PCR
was weak positive that became strongly positive. On combination antibiotic
therapy. . .
DETD [0252] A set of mice were tested for infection with C. ***pneumoniae*** . Of 10 mice tested, 8 (80%) were PCR positive for C. ***pneumoniae*** . The mice were then placed on triple-antibiotic therapy: Amoxicillin, Metronidazole and INH at 50 .mu.g/ml each in their water. Based. . .
DETD [0254] Patents with systemic infections caused by C. ***pneumoniae*** were evaluated for secondary porphyria. The presence of enzymes (i.e., .DELTA.-ALA synthase and PBG deaminase) for heme biosynthesis were determined. . . 24 hours. The results are reported in Table 14.
TABLE 14

Examples of Secondary Porphyria in Patients with Systemic infections caused by C. ***pneumoniae*** .sup.a

	Enzymes of Heme biosynthesis.sup.b			
Patient ID	ALA synthase	PBG deaminase	Elevated Fecal Porphyrins (24 hr) Porphyrin. . .	Elevated Urinary Porphyrins (24 hr)

DETD [0255] Patents with systemic infections caused by C. ***pneumoniae*** were tested for the presence of antibodies to porphyrin ring structures (i.e., vitamin B12, coproporphyrinogen-III, protoporphyrin, porphyrobelinigen and .sup.a-ALA). IgM. . .
DETD . . . reported below in Table 15.
TABLE 15

Examples of Antibody Titers.sup.a to Porphyrin Ring Structures in Patients with Systemic infections caused by C. ***pneumoniae***

Patient B12 Copro III Protoporphyrin Porphyrobelinigen -ALA
ID IgM IgG IgM IgG IgM IgG IgM IgG IgM IgG

KRH 1:640 1:160 1:640 1:160. . .
DETD SEQUENCE CHARACTERISTICS:
LENGTH: 14 amino acids
TYPE: amino acid
STRANDEDNESS: single
TOPOLOGY: linear
MOLECULE TYPE: peptide
SEQUENCE: 100
Cys ***Ile*** ***Gly*** ***Leu*** ***Ala***
Gly ***Thr*** ***Asp*** ***Phe*** ***Ala***
Asn ***Gln*** ***Arg*** ***Pro***
1 5 10
CLM What is claimed is:
. . . each of which is effective against a different phase of chlamydial life cycle, until the biological material is negative for ***Chlamydia*** according to a test that detects elementary body phase ***Chlamydia*** , replicating phase ***Chlamydia*** , and cryptic phase ***Chlamydia*** , thereby treating said asthma.

11. The method of claim 1, wherein the test that detects elementary body phase ***Chlamydia*** , replicating phase ***Chlamydia*** , and cryptic phase ***Chlamydia*** comprises a step of nucleic acid amplification.

. . . for at least 45 days, wherein said antichlamydial agent inhibits infection of cells or inhibits growth or replication of C. ***pneumoniae*** in said mammal, thereby treating said asthma.

L7 ANSWER 2 OF 4 USPATFULL on STN
AN 2003:250524 USPATFULL
TI Identification of antigenic peptide sequences
IN Mitchell, William M., Nashville, TN, UNITED STATES
Stratton, Charles W., Nashville, TN, UNITED STATES
PI US 2003175310 A1 20030918
AI US 2001-20269 A1 20011214 (10)
RLI Continuation of Ser. No. US 1998-25596, filed on 18 Feb 1998, GRANTED, Pat. No. US 6340463 Continuation-in-part of Ser. No. US 1997-911593, filed on 14 Aug 1997, ABANDONED
PRAI US 1996-23921P 19960814 (60)
DT Utility
FS APPLICATION
LREP CLARK & ELBING LLP, 101 FEDERAL STREET, BOSTON, MA, 02110
CLMN Number of Claims: 5
ECL Exemplary Claim: 1
DRWN 4 Drawing Page(s)
LN.CNT 1728
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB Identification of linear amino acid antigenic sequences for the production of both polyclonal and monoclonal antibodies to defined

antigenic domains is described. Also described are antigenic peptides identified by the described methods and antibodies thereto.

SUMM . . . particular embodiments, the larger amino acid sequence is selected from the group consisting of polypeptides expressed by members of the ***Chlamydia*** genus.

DRWD [0008] FIGS. 1A and 1B are sequence alignments of various ***Chlamydia*** MOMPs. Variable domains (VD1-VD4) are boxed. Sequences are aligned with the L2 serovar of C. ***trachomatis*** and are ranked from highest homology (B, D, E, L1) to lower homology (F, C, and A, H, L3). MU is the mouse ***pneumonitis*** C. ***trachomatis*** . PN refers to the human C. ***pneumonia*** . Deletions are indicated by (-). A blank indicates the same residue as L2. The leader sequence is bracketed. Underlined seven. . .

DRWD [0009] FIG. 2 illustrates the predicted antigenic sequences from variable domains 1 (VD1) of various ***Chlamydia*** species. The boxed cysteine (C) residue is not part of the native sequence but has been added at the amino. . .

DRWD [0010] FIG. 3 illustrates the predicted antigenic sequences from variable domain 2 (VD2) of various ***Chlamydia*** species. The boxed cysteine (C) residue is not part of the native seuqence but has been added at the amino. . .

DRWD [0011] FIG. 4 illustrates the predicted antigenic sequences from a common domain of various ***Chlamydia*** species. The shaded box indicates hydrophilic mobile region common to each with expected cross-reactivity for antibodies specific for the sequence.. . .

DETD . . . to inoculation of a vertebrate, particularly a mammal, with a nucleic acid vaccine directed against a pathogenic agent, such as ***Chlamydia*** , resulting in protection of the vertebrate against the pathogenic agent. Representative vertebrates include mice, dogs, cats, chickens, sheep, goats, cows,. . .

DETD . . . spceifically incorporated herein by reference. The teachings of Attorney Docket No. VDB96-02pA2, entitled "Diagnosis and Management of Infection Caused by ***Chlamydia*** " by William M. Mitchell and Charles W. Stratton, filed concurrently with the present application, are also incorporated herein by reference. . .

DETD [0044] 1) Antigenicity of the MOMP (Major Outer Membrane Protein) of ***Chlamydia*** :

DETD [0045] In order to provide ELISA assays that are species- and potentially strain-specific for the various ***Chlamydia*** , two regions in the MOMP have been identified which show minimal amino acid sequence homologies and which are predicted to. . . VD2 which are used similarly to the VD1 sequences. In addition, a highly antigenic domain (FIG. 4) common to all ***Chlamydia*** has been identified and developed as genus-specific ELISA for the ***Chlamydia*** . Immunization of rabbits has verified the antigenicity of each peptide to each peptide (Table 1). Monoclonal antibodies have further verified. .

.

DETD . . . Chlamydiae Identified By Hydrophilicity And Peptide Movement As Highly Antigenic

Chlamydiae Species	Peptide.sup.b	Titer.sup.a Pre	Post
C. ***pneumoniae***	90-105	100	>3200

C. ***trachomatis***	L2	91-106	800	>3200
C. psittaci		92-106	400	>3200
C. ***trachomatis***	(mouse)	89-105	0	>3200
C. ***pneumoniae***		158-171	25	>3200
C. ***trachomatis***	L2	159-175	200	>3200
C. psittaci		160-172	100	>3200
C. ***trachomatis***	(mouse)	158-171	800	>3200
C. ***pneumoniae***		342-354	200	>3200
C. ***trachomatis***	L2	342-354	100	>3200
C. psittaci		ND.sup.c		
C. ***trachomatis***	(mouse)	ND.sup.c		

.sup.aReciprocal titer
.sup.bImmunogenic peptide and ELISA antigen of specific amino acid sequence against the indicated pre-immunization and post-immunization rabbit. .
.

DETD [0047] Table 2 illustrates reciprocal titers of a polyclonal and monoclonal antibody against C. ***trachomatis*** cross-reactive against a C. ***pneumoniae*** peptide encompassing amino acids 342-354 and a recombinant full length MOMP from C. ***pneumoniae*** . Note that the monoclonal antibody raised against C. ***trachomatis*** has as its epitope genus-specific reactivity against peptide 342-354 of C. ***pneumoniae*** .

TABLE 2

	Titer.sup.a	
Antigen	Polyclonal Ab.sup.b	Monoclonal Ab.sup.c
CPN Momp.sup.d	400	0
CPN 90-105.sup.e	50	0
CPN 158-171.sup.f. . .	50	0
CPN 342-354.sup.g	>3200	1600

.sup.aReciprocal titer
.sup.bPolyclonal goat Ab from Chemicon International, Inc. (Temecula, CA) against MOMP of C. ***trachomatis***
.sup.CMonoclonal Ab from ICN Immunologicals (Costa Mesa, CA) against MOMP of C. ***trachomatis***
.sup.dC. ***pneumoniaerecombinant*** MOMP
.sup.eAmino acid peptide 90-105 of C. ***pneumoniae***
.sup.fAmino acid peptide 158-171 of C. ***pneumoniae***
.sup.gAmino acid peptide 342-354 of C. ***pneumoniae***

DETD [0048] 2) Antigenicity of the 76 k D Protein of C. ***Pneumoniae*** :

DETD [0049] C. ***pneumoniae*** expresses a gene encoding a unique 76 kD protein (Perez-Melgosa et al., Infect. Immun. 62:880-886 (1994)). Hydrophilicity/peptide flexibility analysis predicts. . .

DETD [0051] C. ***pneumoniae*** expresses three known genes with significant homology to the human heat shock proteins of 70, 60 and 10 kD. Antigenicity. . . may result in molecular mimicry and autoimmunity. Indeed, it is postulated that the tubal scarring secondary to infection from C. ***trachomatis*** is due to cross-reactive cell mediated immunity against one or more heat shock proteins.

DETD [0052] a) C. ***pneumonia*** DNAK/Heatshock Protein 70:

DETD . . . full length peptide (AA 521-536) and the Chlamydial-specific

epitopic sequence identified as AA 527-536 or truncated for the identification of ***Chlamydia*** -specific antibodies. Table 5 illustrates other potential antigenic sequences for the DNAK protein expressed by C. ***pneumoniae*** based on either peptide flexibility or hydrophilicity and extended to include amino acids found in adjacent hydrophilic or flexible segments, as well as inclusion of aromatic amino acids immediately adjacent to the predicted antigens.

TABLE 4

C. ***pneumoniae*** KEEDKKRREASDAKNE (SEQ ID NO: 11)
(AA 521-536) |||++++| |
human hsp70 AEEDRRKKERVEAVNM (SEQ ID NO: 12)
(AA 569-584)

DETD [0055] b) C. ***pneumoniae*** GROEL/Heatshock Protein (hsp 60) 60:

DETD [0056] Two peptides expressed by the GROEL gene of C. ***pneumoniae*** have a high correlation of hydrophilicity and segment mobility (Table 6). Residues with similar negative charges are identified by "*" symbols. The sequences are highly conserved between C. ***pneumoniae*** heat shock protein (hsp) 60 and the human hsp 60 associated with the mitochondrion. Thus the potential for molecular mimicry. . . well as inclusion of aromatic amino acids immediately adjacent to the predicted areas, are illustrated in Table 7.

TABLE 6

C. ***pneumoniae*** TEIEMKEKKDRVDD (SEQ ID NO: 23)
hsp 60 * | ||||| |
(AA 385-398) SDVEVNEKKDRVTD (SEQ ID NO: 24)
human hsp 60
(AA 410-423)

C. ***pneumoniae*** hsp 60 EDSTSDYDKEK (SEQ ID NO: 25)
hsp 60 * ||*|*|||
(AA 354-364) DVTTSEYEKEK (SEQ ID NO: 26)
human hsp 60
(AA 410-420)

DETD [0058] c) C. ***pneumoniae*** GROES/Heat Shock Protein 10 (hsp 10):

DETD . . . respect to hydrophilicity/peptide movement analysis. Comparison to mouse chaperonin 10 indicates little homology of these bacterial antigenic domains with C. ***pneumoniae*** hsp 10 (Table 8).

TABLE 8

C. ***pneumoniae*** KREEEEATAR (SEQ ID NO: 32)
(AA 20-29) | | +
mouse chaperonin 10 ERSAAETVTK (SEQ ID NO: 33)
(AA 19-28)

C. ***pneumoniae*** DTAKKKQDRAE (SEQ ID NO: 34)
(AA 36-46) * | |
mouse chaperonin 10 EKSQGKVLQAT (SEQ ID NO: 35)
(AA 35-45)

C. ***pneumoniae*** GTGKRTDDGT (SEQ ID NO: 36)

(AA 51-60) ||+ |
mouse chaperonin 10 GSGGKGKSGE (SEQ ID NO: 37)
(AA 50-59)
DETD [0060] 4) Antigenicity of the Crysteine-Rich Proteins of C. ***pneumoniae***
DETD SEQUENCE CHARACTERISTICS:
SEQ ID NO: 1
LENGTH: 14
TYPE: PRT
ORGANISM: ***Chlamydia*** ***pneumoniae***
SEQUENCE: 1
Lys Pro Lys Glu Ser Lys Thr Asp Ser Val Glu Arg Trp Ser
1 5 10
DETD SEQUENCE CHARACTERISTICS:
SEQ ID NO: 2
LENGTH: 11
TYPE: PRT
ORGANISM: ***Chlamydia*** ***pneumoniae***
SEQUENCE: 2
Ser Ser Asn Ser Ser Ser Ser Thr Ser Arg Ser
1 5 10
DETD SEQUENCE CHARACTERISTICS:
SEQ ID NO: 3
LENGTH: 8
TYPE: PRT
ORGANISM: ***Chlamydia*** ***pneumoniae***
SEQUENCE: 3
Gly Ser Lys Gln Gln Gly Ser Ser
1 5
DETD SEQUENCE CHARACTERISTICS:
SEQ ID NO: 4
LENGTH: 8
TYPE: PRT
ORGANISM: ***Chlamydia*** ***pneumoniae***
SEQUENCE: 4
Gly Lys Ala Gly Gln Gln Gln Gly
1 5
DETD SEQUENCE CHARACTERISTICS:
SEQ ID NO: 5
LENGTH: 9
TYPE: PRT
ORGANISM: ***Chlamydia*** ***pneumoniae***
SEQUENCE: 5
Pro Ser Glu Thr Ser Thr Thr Glu Lys
1 5
DETD SEQUENCE CHARACTERISTICS:
SEQ ID NO: 6
LENGTH: 8
TYPE: PRT
ORGANISM: ***Chlamydia*** ***pneumoniae***
SEQUENCE: 6
Lys Pro Ala Asp Gly Ser Asp Val
1 5
DETD SEQUENCE CHARACTERISTICS:
SEQ ID NO: 7

LENGTH: 11
TYPE: PRT
ORGANISM: ***Chlamydia*** ***pneumoniae***
SEQUENCE: 7
Asn Gly Gln Lys Lys Pro Leu Tyr Leu Tyr Gly
1 5 10
DETD SEQUENCE CHARACTERISTICS:
SEQ ID NO: 8
LENGTH: 19
TYPE: PRT
ORGANISM: ***Chlamydia*** ***pneumoniae***
SEQUENCE: 8
Ser Asp Val Pro Asn Pro Gly Thr Thr Val Gly Gly Ser Lys Gln Gln
1 5. . .
DETD SEQUENCE CHARACTERISTICS:
SEQ ID NO: 9
LENGTH: 15
TYPE: PRT
ORGANISM: ***Chlamydia*** ***pneumoniae***
SEQUENCE: 9
His Met Phe Asn Thr Glu Asn Pro Asp Ser Gln Ala Ala Gln Gln
1 5 10. . .
DETD SEQUENCE CHARACTERISTICS:
SEQ ID NO: 10
LENGTH: 9
TYPE: PRT
ORGANISM: ***Chlamydia*** ***pneumoniae***
SEQUENCE: 10
Asp Asp Ala Glu Asn Glu Thr Ala Ser
1 5
DETD SEQUENCE CHARACTERISTICS:
SEQ ID NO: 11
LENGTH: 16
TYPE: PRT
ORGANISM: ***Chlamydia*** ***pneumoniae***
SEQUENCE: 11
Lys Glu Glu Asp Lys Lys Arg Arg Glu Ala Ser Asp Ala Lys Asn Glu
1 5. . .
DETD SEQUENCE CHARACTERISTICS:
SEQ ID NO: 13
LENGTH: 22
TYPE: PRT
ORGANISM: ***Chlamydia*** ***pneumoniae***
SEQUENCE: 13
Lys Lys His Ser Phe Ser Thr Lys Pro Pro Ser Asn Asn Gly Ser Ser
1 5. . .
DETD SEQUENCE CHARACTERISTICS:
SEQ ID NO: 14
LENGTH: 14
TYPE: PRT
ORGANISM: ***Chlamydia*** ***pneumoniae***
SEQUENCE: 14
Tyr Thr Val Thr Ser Gly Ser Lys Gly Asp Ala Val Phe Glu
1 5 10
DETD SEQUENCE CHARACTERISTICS:

SEQ ID NO: 15
LENGTH: 11
TYPE: PRT
ORGANISM: ***Chlamydia*** ***pneumoniae***
SEQUENCE: 15
Thr Ser Ser Glu Gly Thr Arg Thr Thr Pro Ser
1 5 10
DETD SEQUENCE CHARACTERISTICS:
SEQ ID NO: 16
LENGTH: 8
TYPE: PRT
ORGANISM: ***Chlamydia*** ***pneumoniae***
SEQUENCE: 16
Ser Glu His Lys Lys Ser Ser Lys
1 5
DETD SEQUENCE CHARACTERISTICS:
SEQ ID NO: 17
LENGTH: 14
TYPE: PRT
ORGANISM: ***Chlamydia*** ***pneumoniae***
SEQUENCE: 17
Lys Asp Val Ala Ser Gly Lys Glu Gln Lys Ile Arg Ile Glu
1 5 10
DETD SEQUENCE CHARACTERISTICS:
SEQ ID NO: 18
LENGTH: 16
TYPE: PRT
ORGANISM: ***Chlamydia*** ***pneumoniae***
SEQUENCE: 18
Glu Arg Asn Thr Thr Ile Pro Thr Gln Lys Lys Gln Ile Phe Ser Thr
1 5. . .
DETD SEQUENCE CHARACTERISTICS:
SEQ ID NO: 19
LENGTH: 16
TYPE: PRT
ORGANISM: ***Chlamydia*** ***pneumoniae***
SEQUENCE: 19
Tyr Phe Asn Asp Ser Gln Arg Ala Ser Ser Thr Lys Asp Ala Gly Arg
1 5. . .
DETD SEQUENCE CHARACTERISTICS:
SEQ ID NO: 20
LENGTH: 15
TYPE: PRT
ORGANISM: ***Chlamydia*** ***pneumoniae***
SEQUENCE: 20
Glu Glu Phe Lys Lys Gln Glu Gly Ile Asp Leu Ser Lys Asp Asn
1 5 10. . .
DETD SEQUENCE CHARACTERISTICS:
SEQ ID NO: 21
LENGTH: 12
TYPE: PRT
ORGANISM: ***Chlamydia*** ***pneumoniae***
SEQUENCE: 21
Asn Ala Lys Gly Gly Pro Asn Ile Asn Thr Glu Asp
1 5 10

DETD SEQUENCE CHARACTERISTICS:
SEQ ID NO: 22
LENGTH: 16
TYPE: PRT
ORGANISM: ***Chlamydia*** ***pneumoniae***
SEQUENCE: 22
Gly Glu Arg Pro Met Ala Lys Asp Asn Lys Glu Ile Gly Arg Phe Asp
1 5. . .
DETD SEQUENCE CHARACTERISTICS:
SEQ ID NO: 23
LENGTH: 14
TYPE: PRT
ORGANISM: ***Chlamydia*** ***pneumoniae***
SEQUENCE: 23
Thr Glu Ile Glu Met Lys Glu Lys Lys Asp Arg Val Asp Asp
1 5 10
DETD SEQUENCE CHARACTERISTICS:
SEQ ID NO: 25
LENGTH: 11
TYPE: PRT
ORGANISM: ***Chlamydia*** ***pneumoniae***
SEQUENCE: 25
Glu Asp Ser Thr Ser Asp Tyr Asp Lys Glu Lys
1 5 10
DETD SEQUENCE CHARACTERISTICS:
SEQ ID NO: 27
LENGTH: 7
TYPE: PRT
ORGANISM: ***Chlamydia*** ***pneumoniae***
SEQUENCE: 27
Asp Asp Lys Ser Ser Ser Ala
1 5
DETD SEQUENCE CHARACTERISTICS:
SEQ ID NO: 28
LENGTH: 15
TYPE: PRT
ORGANISM: ***Chlamydia*** ***pneumoniae***
SEQUENCE: 28
Lys Lys Gln Ile Glu Asp Ser Thr Ser Asp Tyr Val Ser Glu Glu
1 5 10. . .
DETD SEQUENCE CHARACTERISTICS:
SEQ ID NO: 29
LENGTH: 12
TYPE: PRT
ORGANISM: ***Chlamydia*** ***pneumoniae***
SEQUENCE: 29
Ser Ser Tyr Phe Ser Thr Asn Pro Glu Thr Gln Glu
1 5 10
DETD SEQUENCE CHARACTERISTICS:
SEQ ID NO: 30
LENGTH: 16
TYPE: PRT
ORGANISM: ***Chlamydia*** ***pneumoniae***
SEQUENCE: 30
Glu Lys Val Gly Lys Asn Gly Ser Ile Thr Val Glu Glu Ala Asp Lys

1 5. . .
DETD SEQUENCE CHARACTERISTICS:
SEQ ID NO: 31
LENGTH: 15
TYPE: PRT
ORGANISM: ***Chlamydia*** ***pneumoniae***
SEQUENCE: 31
Ser Lys Thr Ala Asp Lys Ala Gly Asp Gly Thr Thr Thr Ala Thr
1 5 10. . .
DETD SEQUENCE CHARACTERISTICS:
SEQ ID NO: 32
LENGTH: 10
TYPE: PRT
ORGANISM: ***Chlamydia*** ***pneumoniae***
SEQUENCE: 32
Lys Arg Glu Glu Glu Glu Ala Thr Ala Arg
1 5 10
DETD SEQUENCE CHARACTERISTICS:
SEQ ID NO: 34
LENGTH: 11
TYPE: PRT
ORGANISM: ***Chlamydia*** ***pneumoniae***
SEQUENCE: 34
Asp Thr Ala Lys Lys Lys Gln Asp Arg Ala Glu
1 5 10
DETD SEQUENCE CHARACTERISTICS:
SEQ ID NO: 36
LENGTH: 10
TYPE: PRT
ORGANISM: ***Chlamydia*** ***pneumoniae***
SEQUENCE: 36
Gly Thr Gly Lys Arg Thr Asp Asp Gly Thr
1 5 10
DETD SEQUENCE CHARACTERISTICS:
SEQ ID NO: 38
LENGTH: 25
TYPE: PRT
ORGANISM: ***Chlamydia*** ***pneumoniae***
SEQUENCE: 38
Arg Arg Asn Lys Gln Pro Val Glu Gln Lys Ser Arg Gly Ala Phe Cys
1 5. . .
DETD SEQUENCE CHARACTERISTICS:
SEQ ID NO: 39
LENGTH: 19
TYPE: PRT
ORGANISM: ***Chlamydia*** ***pneumoniae***
SEQUENCE: 39
Asp Met Arg Pro Gly Asp Lys Lys Val Phe Thr Val Glu Phe Cys Pro
1 5. . .
DETD SEQUENCE CHARACTERISTICS:
SEQ ID NO: 40
LENGTH: 19
TYPE: PRT
ORGANISM: ***Chlamydia*** ***pneumoniae***
SEQUENCE: 40

Ser Ser Asp Pro Glu Thr Thr Pro Thr Ser Asp Gly Lys Val Trp Lys
1 5. . .
DETD SEQUENCE CHARACTERISTICS:
SEQ ID NO: 41
LENGTH: 20
TYPE: PRT
ORGANISM: ***Chlamydia*** ***pneumoniae***
SEQUENCE: 41
Thr Ser Glu Ser Asn Cys Gly Thr Cys Thr Ser Cys Ala Glu Thr Thr
1 5. . .
DETD SEQUENCE CHARACTERISTICS:
SEQ ID NO: 42
LENGTH: 11
TYPE: PRT
ORGANISM: ***Chlamydia*** ***pneumoniae***
SEQUENCE: 42
Lys Leu Gly Ser Lys Glu Ser Val Glu Phe Ser
1 5 10
DETD SEQUENCE CHARACTERISTICS:
SEQ ID NO: 43
LENGTH: 16
TYPE: PRT
ORGANISM: ***Chlamydia*** ***pneumoniae***
SEQUENCE: 43
Thr Val Tyr Arg Ile Cys Val Thr Asn Arg Gly Ser Ala Glu Asp Thr
1 5. . .
DETD SEQUENCE CHARACTERISTICS:
SEQ ID NO: 44
LENGTH: 11
TYPE: PRT
ORGANISM: ***Chlamydia*** ***pneumoniae***
SEQUENCE: 44
Glu Tyr Ser Ile Ser Val Ser Asn Pro Gly Asp
1 5 10
DETD SEQUENCE CHARACTERISTICS:
SEQ ID NO: 45
LENGTH: 100
TYPE: PRT
ORGANISM: ***Chlamydia*** ***trachomatis***
SEQUENCE: 45
Met Lys Lys Leu Leu Lys Ser Val Leu Val Phe Ala Ala Leu Ser Ser
1 5. . .
DETD SEQUENCE CHARACTERISTICS:
SEQ ID NO: 46
LENGTH: 100
TYPE: PRT
ORGANISM: ***Chlamydia*** ***trachomatis***
SEQUENCE: 46
Met Lys Lys Leu Leu Lys Ser Val Leu Val Phe Ala Ala Leu Ser Ser
1 5. . .
DETD SEQUENCE CHARACTERISTICS:
SEQ ID NO: 47
LENGTH: 100
TYPE: PRT
ORGANISM: ***Chlamydia*** ***trachomatis***

SEQUENCE: 47
Met Lys Lys Leu Leu Lys Ser Val Leu Val Phe Ala Ala Leu Ser Ser
1 5. . .
DETD SEQUENCE CHARACTERISTICS:
SEQ ID NO: 48
LENGTH: 100
TYPE: PRT
ORGANISM: ***Chlamydia*** ***trachomatis***
SEQUENCE: 48
Met Lys Lys Leu Leu Lys Ser Val Leu Val Phe Ala Ala Leu Ser Ser
1 5. . .
DETD SEQUENCE CHARACTERISTICS:
SEQ ID NO: 49
LENGTH: 100
TYPE: PRT
ORGANISM: ***Chlamydia*** ***trachomatis***
SEQUENCE: 49
Met Lys Lys Leu Leu Lys Ser Val Leu Val Phe Ala Ala Leu Ser Ser
1 5. . .
DETD SEQUENCE CHARACTERISTICS:
SEQ ID NO: 50
LENGTH: 101
TYPE: PRT
ORGANISM: ***Chlamydia*** ***trachomatis***
SEQUENCE: 50
Met Lys Lys Leu Leu Lys Ser Val Leu Val Phe Ala Ala Leu Ser Ser
1 5. . .
DETD SEQUENCE CHARACTERISTICS:
SEQ ID NO: 51
LENGTH: 103
TYPE: PRT
ORGANISM: ***Chlamydia*** ***trachomatis***
SEQUENCE: 51
Met Lys Lys Leu Leu Lys Ser Val Leu Val Phe Ala Ala Leu Ser Ser
1 5. . .
DETD SEQUENCE CHARACTERISTICS:
SEQ ID NO: 52
LENGTH: 102
TYPE: PRT
ORGANISM: ***Chlamydia*** ***trachomatis***
SEQUENCE: 52
Met Lys Lys Leu Leu Lys Ser Val Leu Val Phe Ala Ala Leu Ser Ser
1 5. . .
DETD SEQUENCE CHARACTERISTICS:
SEQ ID NO: 53
LENGTH: 97
TYPE: PRT
ORGANISM: ***Chlamydia*** ***trachomatis***
SEQUENCE: 53
Met Lys Lys Leu Leu Lys Ser Val Leu Val Phe Ala Ala Leu Ser Ser
1 5. . .
DETD SEQUENCE CHARACTERISTICS:
SEQ ID NO: 54
LENGTH: 102
TYPE: PRT

ORGANISM: ***Chlamydia*** ***trachomatis***
SEQUENCE: 54
Met Lys Lys Leu Leu Lys Ser Val Leu Val Phe Ala Ala Leu Ser Ser
1 5. . .
DETD SEQUENCE CHARACTERISTICS:
SEQ ID NO: 55
LENGTH: 100
TYPE: PRT
ORGANISM: ***Chlamydia*** ***trachomatis***
SEQUENCE: 55
Met Lys Lys Leu Leu Lys Ser Val Ala Val Phe Val Ala Gly Ser Ser
1 5. . .
DETD SEQUENCE CHARACTERISTICS:
SEQ ID NO: 56
LENGTH: 99
TYPE: PRT
ORGANISM: ***Chlamydia*** ***pneumoniae***
SEQUENCE: 56
Met Lys Lys Leu Leu Lys Ala Val Leu Ala Phe Ala Phe Ala Gly Ser
1 5. . .
DETD SEQUENCE CHARACTERISTICS:
SEQ ID NO: 57
LENGTH: 100
TYPE: PRT
ORGANISM: ***Chlamydia*** ***trachomatis***
SEQUENCE: 57
Cys Thr Ala Arg Glu Asn Pro Ala Tyr Gly Arg His Met Gln Asp Ala
1 5. . .
DETD SEQUENCE CHARACTERISTICS:
SEQ ID NO: 58
LENGTH: 65
TYPE: PRT
ORGANISM: ***Chlamydia*** ***trachomatis***
SEQUENCE: 58
Leu Thr Ala Arg Glu Asn Pro Ala Tyr Gly Arg His Met Gln Asp Ala
1 5. . .
DETD SEQUENCE CHARACTERISTICS:
SEQ ID NO: 59
LENGTH: 134
TYPE: PRT
ORGANISM: ***Chlamydia*** ***trachomatis***
SEQUENCE: 59
Cys Thr Ala Arg Glu Asn Pro Ala Tyr Gly Arg His Met Gln Asp Ala
1 5. . .
DETD SEQUENCE CHARACTERISTICS:
SEQ ID NO: 60
LENGTH: 99
TYPE: PRT
ORGANISM: ***Chlamydia*** ***trachomatis***
SEQUENCE: 60
Leu Thr Ala Arg Glu Asn Pro Ala Tyr Gly Arg His Met Gln Asp Ala
1 5. . .
DETD SEQUENCE CHARACTERISTICS:
SEQ ID NO: 61
LENGTH: 99

TYPE: PRT
ORGANISM: ***Chlamydia*** ***trachomatis***
SEQUENCE: 61
Cys Thr Ala Arg Glu Asn Pro Ala Tyr Gly Arg His Met Gln Asp Ala
1 5. . .
DETD SEQUENCE CHARACTERISTICS:
SEQ ID NO: 62
LENGTH: 99
TYPE: PRT
ORGANISM: ***Chlamydia*** ***trachomatis***
SEQUENCE: 62
Leu Val Glu Arg Thr Asn Pro Ala Tyr Gly Lys His Met Gln Asp Ala
1 5. . .
DETD SEQUENCE CHARACTERISTICS:
SEQ ID NO: 63
LENGTH: 100
TYPE: PRT
ORGANISM: ***Chlamydia*** ***trachomatis***
SEQUENCE: 63
Asn Val Ala Arg Pro Asn Pro Ala Tyr Gly Lys His Met Gln Asp Ala
1 5. . .
DETD SEQUENCE CHARACTERISTICS:
SEQ ID NO: 64
LENGTH: 100
TYPE: PRT
ORGANISM: ***Chlamydia*** ***trachomatis***
SEQUENCE: 64
Asn Val Ala Arg Pro Asn Pro Ala Tyr Gly Lys His Met Gln Asp Ala
1 5. . .
DETD SEQUENCE CHARACTERISTICS:
SEQ ID NO: 65
LENGTH: 100
TYPE: PRT
ORGANISM: ***Chlamydia*** ***trachomatis***
SEQUENCE: 65
Asn Val Ala Arg Pro Asn Pro Ala Tyr Gly Lys His Met Gln Asp Ala
1 5. . .
DETD SEQUENCE CHARACTERISTICS:
SEQ ID NO: 66
LENGTH: 100
TYPE: PRT
ORGANISM: ***Chlamydia*** ***trachomatis***
SEQUENCE: 66
Asn Val Ala Arg Pro Asn Pro Ala Tyr Gly Lys His Met Gln Asp Ala
1 5. . .
DETD SEQUENCE CHARACTERISTICS:
SEQ ID NO: 67
LENGTH: 96
TYPE: PRT
ORGANISM: ***Chlamydia*** ***trachomatis***
SEQUENCE: 67
Ala Ser Arg Glu Asn Pro Ala Tyr Gly Lys His Met Gln Asp Ala Glu
1 5. . .
DETD SEQUENCE CHARACTERISTICS:
SEQ ID NO: 68

LENGTH: 100
TYPE: PRT
ORGANISM: ***Chlamydia*** ***pneumoniae***
SEQUENCE: 68
Tyr Thr Thr Ala Val Asp Arg Pro Asn Pro Ala Tyr Asn Lys His Leu
1 5. . .
DETD SEQUENCE CHARACTERISTICS:
SEQ ID NO: 69
LENGTH: 100
TYPE: PRT
ORGANISM: ***Chlamydia*** ***trachomatis***
SEQUENCE: 69
Ala Leu Trp Glu Cys Gly Cys Ala Thr Leu Gly Ala Ser Phe Gln Tyr
1 5. . .
DETD SEQUENCE CHARACTERISTICS:
SEQ ID NO: 70
LENGTH: 100
TYPE: PRT
ORGANISM: ***Chlamydia*** ***trachomatis***
SEQUENCE: 70
Ala Leu Trp Glu Cys Gly Cys Ala Thr Leu Gly Ala Ser Phe Gln Tyr
1 5. . .
DETD SEQUENCE CHARACTERISTICS:
SEQ ID NO: 71
LENGTH: 100
TYPE: PRT
ORGANISM: ***Chlamydia*** ***trachomatis***
SEQUENCE: 71
Ala Leu Trp Glu Cys Gly Cys Ala Thr Leu Gly Ala Ser Phe Gln Tyr
1 5. . .
DETD SEQUENCE CHARACTERISTICS:
SEQ ID NO: 72
LENGTH: 100
TYPE: PRT
ORGANISM: ***Chlamydia*** ***trachomatis***
SEQUENCE: 72
Ala Leu Trp Glu Cys Gly Cys Ala Thr Leu Gly Ala Ser Phe Gln Tyr
1 5. . .
DETD SEQUENCE CHARACTERISTICS:
SEQ ID NO: 73
LENGTH: 100
TYPE: PRT
ORGANISM: ***Chlamydia*** ***trachomatis***
SEQUENCE: 73
Ala Leu Trp Glu Cys Gly Cys Ala Thr Leu Gly Ala Ser Phe Gln Tyr
1 5. . .
DETD SEQUENCE CHARACTERISTICS:
SEQ ID NO: 74
LENGTH: 100
TYPE: PRT
ORGANISM: ***Chlamydia*** ***trachomatis***
SEQUENCE: 74
Ala Leu Trp Glu Cys Gly Cys Ala Thr Leu Gly Ala Ser Phe Gln Tyr
1 5. . .
DETD SEQUENCE CHARACTERISTICS:

SEQ ID NO: 75
LENGTH: 100
TYPE: PRT
ORGANISM: ***Chlamydia*** ***trachomatis***
SEQUENCE: 75
Ala Leu Trp Glu Cys Gly Cys Ala Thr Leu Gly Ala Ser Phe Gln Tyr
1 5. . .
DETD SEQUENCE CHARACTERISTICS:
SEQ ID NO: 76
LENGTH: 100
TYPE: PRT
ORGANISM: ***Chlamydia*** ***trachomatis***
SEQUENCE: 76
Ala Leu Trp Glu Cys Gly Cys Ala Thr Leu Gly Ala Ser Phe Gln Tyr
1 5. . .
DETD SEQUENCE CHARACTERISTICS:
SEQ ID NO: 77
LENGTH: 100
TYPE: PRT
ORGANISM: ***Chlamydia*** ***trachomatis***
SEQUENCE: 77
Ala Leu Trp Glu Cys Gly Cys Ala Thr Leu Gly Ala Ser Phe Gln Tyr
1 5. . .
DETD SEQUENCE CHARACTERISTICS:
SEQ ID NO: 78
LENGTH: 100
TYPE: PRT
ORGANISM: ***Chlamydia*** ***trachomatis***
SEQUENCE: 78
Ala Leu Trp Glu Cys Gly Cys Ala Thr Leu Gly Ala Ser Phe Gln Tyr
1 5. . .
DETD SEQUENCE CHARACTERISTICS:
SEQ ID NO: 79
LENGTH: 100
TYPE: PRT
ORGANISM: ***Chlamydia*** ***trachomatis***
SEQUENCE: 79
Ala Leu Trp Glu Cys Gly Cys Ala Thr Leu Gly Ala Ser Phe Gln Tyr
1 5. . .
DETD SEQUENCE CHARACTERISTICS:
SEQ ID NO: 80
LENGTH: 100
TYPE: PRT
ORGANISM: ***Chlamydia*** ***pneumoniae***
SEQUENCE: 80
Gly Leu Trp Glu Cys Gly Cys Ala Thr Leu Gly Glu Ser Phe Gln Tyr
1 5. . .
DETD SEQUENCE CHARACTERISTICS:
SEQ ID NO: 81
LENGTH: 94
TYPE: PRT
ORGANISM: ***Chlamydia*** ***trachomatis***
SEQUENCE: 81
Asp Thr Ile Arg Ile Ala Gln Pro Lys Ser Ala Thr Thr Val Phe Asp
1 5. . .

DETD SEQUENCE CHARACTERISTICS:
SEQ ID NO: 82
LENGTH: 92
TYPE: PRT
ORGANISM: ***Chlamydia*** ***trachomatis***
SEQUENCE: 82
Asp Thr Ile Arg Ile Ala Gln Pro Lys Ser Ala Glu Thr Ile Phe Asp
1 5. . .
DETD SEQUENCE CHARACTERISTICS:
SEQ ID NO: 83
LENGTH: 94
TYPE: PRT
ORGANISM: ***Chlamydia*** ***trachomatis***
SEQUENCE: 83
Asp Thr Ile Arg Ile Ala Gln Pro Lys Ser Ala Thr Ala Ile Phe Asp
1 5. . .
DETD SEQUENCE CHARACTERISTICS:
SEQ ID NO: 84
LENGTH: 94
TYPE: PRT
ORGANISM: ***Chlamydia*** ***trachomatis***
SEQUENCE: 84
Asp Thr Ile Arg Ile Ala Gln Pro Lys Ser Ala Thr Ala Ile Phe Asp
1 5. . .
DETD SEQUENCE CHARACTERISTICS:
SEQ ID NO: 85
LENGTH: 94
TYPE: PRT
ORGANISM: ***Chlamydia*** ***trachomatis***
SEQUENCE: 85
Asp Thr Ile Arg Ile Ala Gln Pro Lys Leu Ala Thr Ala Ile Phe Asp
1 5. . .
DETD SEQUENCE CHARACTERISTICS:
SEQ ID NO: 86
LENGTH: 95
TYPE: PRT
ORGANISM: ***Chlamydia*** ***trachomatis***
SEQUENCE: 86
Asp Thr Ile Arg Ile Ala Gln Pro Arg Leu Val Thr Pro Val Val Asp
1 5. . .
DETD SEQUENCE CHARACTERISTICS:
SEQ ID NO: 87
LENGTH: 95
TYPE: PRT
ORGANISM: ***Chlamydia*** ***trachomatis***
SEQUENCE: 87
Asp Thr Ile Arg Ile Ala Gln Pro Lys Leu Ala Glu Ala Ile Leu Asp
1 5. . .
DETD SEQUENCE CHARACTERISTICS:
SEQ ID NO: 88
LENGTH: 94
TYPE: PRT
ORGANISM: ***Chlamydia*** ***trachomatis***
SEQUENCE: 88
Asp Thr Ile Arg Ile Ala Gln Pro Lys Leu Ala Lys Pro Val Leu Asp

1 5. . .
DETD SEQUENCE CHARACTERISTICS:
SEQ ID NO: 89
LENGTH: 94
TYPE: PRT
ORGANISM: ***Chlamydia*** ***trachomatis***
SEQUENCE: 89
Asp Thr Ile Arg Ile Ala Gln Pro Lys Leu Ala Glu Ala Ile Leu Asp
1 5. . .
DETD SEQUENCE CHARACTERISTICS:
SEQ ID NO: 90
LENGTH: 95
TYPE: PRT
ORGANISM: ***Chlamydia*** ***trachomatis***
SEQUENCE: 90
Asp Thr Ile Arg Ile Ala Gln Pro Lys Leu Ala Glu Ala Val Leu Asp
1 5. . .
DETD SEQUENCE CHARACTERISTICS:
SEQ ID NO: 91
LENGTH: 91
TYPE: PRT
ORGANISM: ***Chlamydia*** ***trachomatis***
SEQUENCE: 91
Asp Thr Ile Arg Ile Ala Gln Pro Lys Leu Glu Thr Ser Ile Leu Lys
1 5. . .
DETD SEQUENCE CHARACTERISTICS:
SEQ ID NO: 92
LENGTH: 93
TYPE: PRT
ORGANISM: ***Chlamydia*** ***pneumoniae***
SEQUENCE: 92
Asp Asn Ile Arg Ile Ala Gln Pro Lys Leu Pro Thr Ala Val Leu Asn
1 5. . .
DETD SEQUENCE CHARACTERISTICS:
SEQ ID NO: 93
LENGTH: 17
TYPE: PRT
ORGANISM: ***Chlamydia*** ***pneumoniae***
SEQUENCE: 93
Cys Thr Gly Ser Ala Ala Ala Asn Tyr Thr Thr Ala Val Asp Arg Pro
1 5. . .
DETD SEQUENCE CHARACTERISTICS:
SEQ ID NO: 94
LENGTH: 18
TYPE: PRT
ORGANISM: ***Chlamydia*** ***trachomatis***
SEQUENCE: 94
Cys Thr Gly Asp Ala Asp Leu Thr Thr Ala Pro Thr Pro Ala Ser Arg
1 5. . .
DETD SEQUENCE CHARACTERISTICS:
SEQ ID NO: 95
LENGTH: 19
TYPE: PRT
ORGANISM: ***Chlamydia*** ***trachomatis***
SEQUENCE: 95

Cys Thr Thr Ala Thr Gly Asn Ala Ala Ala Pro Ser Thr Cys Thr Ala
1 5. . .
DETD SEQUENCE CHARACTERISTICS:
SEQ ID NO: 96
LENGTH: 17
TYPE: PRT
ORGANISM: ***Chlamydia*** psitacci
SEQUENCE: 96
Cys Ala Ser Gly Thr Ala Ser Asn Thr Thr Val Ala Ala Asp Arg Ser
1. . .
DETD SEQUENCE CHARACTERISTICS:
SEQ ID NO: 97
LENGTH: 15
TYPE: PRT
ORGANISM: ***Chlamydia*** ***pneumoniae***
SEQUENCE: 97
Cys Phe Gly Val Lys Gly Thr Thr Val Asn Ala Asn Glu Leu Pro
1 5 10. . .
DETD SEQUENCE CHARACTERISTICS:
SEQ ID NO: 98
LENGTH: 15
TYPE: PRT
ORGANISM: ***Chlamydia*** ***trachomatis***
SEQUENCE: 98
Cys Phe Gly Arg Asp Glu Thr Ala Val Ala Ala Asp Asp Ile Pro
1 5 10. . .
DETD SEQUENCE CHARACTERISTICS:
SEQ ID NO: 99
LENGTH: 18
TYPE: PRT
ORGANISM: ***Chlamydia*** ***trachomatis***
SEQUENCE: 99
Cys Phe Gly Asp Asn Glu Asn His Ala Thr Val Ser Asp Ser Lys Leu
1 5. . .
DETD SEQUENCE CHARACTERISTICS:
SEQ ID NO: 100
LENGTH: 14
TYPE: PRT
ORGANISM: ***Chlamydia*** psitacci
SEQUENCE: 100
Cys ***Ile*** ***Gly*** ***Leu*** ***Ala***
Gly ***Thr*** ***Asp*** ***Phe*** ***Ala***
Asn ***Gln*** ***Arg*** ***Pro***
1 5 10
DETD SEQUENCE CHARACTERISTICS:
SEQ ID NO: 101
LENGTH: 13
TYPE: PRT
ORGANISM: ***Chlamydia*** ***pneumoniae***
SEQUENCE: 101
Cys Gln Ile Asn Lys Phe Lys Ser Arg Lys Ala Cys Gly
1 5 10
DETD SEQUENCE CHARACTERISTICS:
SEQ ID NO: 102
LENGTH: 13

TYPE: PRT
ORGANISM: ***Chlamydia*** ***trachomatis***
SEQUENCE: 102
Cys Gln Ile Asn Lys Met Lys Ser Arg Phe Ala Cys Gly
1 5 10
DETD SEQUENCE CHARACTERISTICS:
SEQ ID NO: 103
LENGTH: 13
TYPE: PRT
ORGANISM: ***Chlamydia*** ***trachomatis***
SEQUENCE: 103
Cys Gln Leu Asn Lys Met Lys Ser Arg Lys Ala Cys Gly
1 5 10
DETD SEQUENCE CHARACTERISTICS:
SEQ ID NO: 104
LENGTH: 13
TYPE: PRT
ORGANISM: ***Chlamydia*** psitacci
SEQUENCE: 104
Cys Gln Ile Asn Lys Phe Lys Ser Arg Phe Ala Cys Gly
1 5 10
DETD SEQUENCE CHARACTERISTICS:
SEQ ID NO: 105
LENGTH: 6
TYPE: PRT
ORGANISM: ***Chlamydia*** ***pneumoniae***
SEQUENCE: 105
Arg Lys Lys Glu Arg Ser
1 5
DETD SEQUENCE CHARACTERISTICS:
SEQ ID NO: 106
LENGTH: 10
TYPE: PRT
ORGANISM: ***Chlamydia*** ***pneumoniae***
SEQUENCE: 106
Ser Thr Glu Cys Asn Ser Gln Ser Pro Gln
1 5 10

L7 ANSWER 3 OF 4 USPATFULL on STN
AN 2003:244934 USPATFULL
TI Diagnosis and management of infection caused by ***Chlamydia***
IN Mitchell, William M., Nashville, TN, UNITED STATES
Stratton, Charles W., Nashville, TN, UNITED STATES
PI US 2003171348 A1 20030911
US 6664239 B2 20031216
AI US 2002-100785 A1 20020319 (10)
RLI Continuation of Ser. No. US 1998-73661, filed on 6 May 1998, PENDING
Continuation-in-part of Ser. No. US 1998-25521, filed on 18 Feb 1998,
ABANDONED Continuation-in-part of Ser. No. US 1997-911593, filed on 14
Aug 1997, ABANDONED
PRAI US 1997-45739P 19970506 (60)
US 1997-45779P 19970506 (60)
US 1997-45780P 19970506 (60)
US 1997-45784P 19970506 (60)
US 1997-45787P 19970506 (60)

US 1997-45689P 19970506 (60)
DT Utility
FS APPLICATION
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CLMN Number of Claims: 43
ECL Exemplary Claim: 1
DRWN 4 Drawing Page(s)
LN.CNT 4871
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The present invention provides a unique approach for the diagnosis and management of infections by ***Chlamydia*** species, particularly C. ***pneumoniae*** . The invention is based, in part, upon the discovery that a combination of agents directed toward the various stages of the chlamydial life cycle is effective in substantially reducing infection. Products comprising combination of antichlamydial agents, novel compositions and pharmaceutical packs are also described.
TI Diagnosis and management of infection caused by ***Chlamydia***
AB The present invention provides a unique approach for the diagnosis and management of infections by ***Chlamydia*** species, particularly C. ***pneumoniae*** . The invention is based, in part, upon the discovery that a combination of agents directed toward the various stages of. .
.
SUMM . . . members of the genus Chlamydiae induces a significant inflammatory response at the cellular level. For example, genital lesions produced by ***Chlamydia*** ***trachomatis*** frequently elicit a vigorous influx of lymphocytes, macrophages, and plasma cells, suggesting the development of humoral and cellular immunity. Yet, clinically, the initial infection is frequently varied in symptomatology and may even be asymptomatic. Once fully established, the ***Chlamydia*** are difficult to eradicate, with frequent relapse following antibiotic therapy. Evidence also indicates that the ***Chlamydia*** may become dormant and are then shed in quantities too few to reliably detect by culture.
SUMM [0005] ***Chlamydia*** ***pneumoniae*** (hereinafter "C. ***pneumoniae*** ") is the most recent addition to the genus Chlamydiae and is isolated from humans and currently is recognized as causing approximately 10 percent of community acquired cases of ***pneumonia*** (Grayston et al., J. Inf. Dis. 161:618-625 (1990)). This newly recognized pathogen commonly infects the upper and lower respiratory tract and is now recognized as ubiquitous in humans. C. ***pneumoniae*** is well-accepted as a human pathogen that may be difficult to eradicate by standard antibiotic therapy (Hammerschlag et al., Clin. Infect. Dis. 14:178-182 (1992)). C. ***pneumoniae*** is known to persist as a silent or mildly symptomatic pathogen, resulting in a chronic, persistent infection (J. Schacter, In: Baun A L, eg. Microbiology of ***Chlamydia*** , Boca Raton, Fla., CRC Press, 1988, pp. 153-165).
SUMM [0006] The current therapy for suspected/confirmed C. ***pneumoniae*** infection is with a short course (e.g., 2-3 weeks) of a single antibiotic. C. ***pneumoniae*** is susceptible in vitro to tetracyline, erythromycin, clarithromycin, and fluoroquinolones such as ofloxacin and sparfloxacin (Kuo et al., Antimicrob Agents. . . Agents Chemother 38:1873-1878 (1994); M. R. Hammerschlag, Infect. Med. pp. 64-71 (1994)). Despite this demonstration of in vitro susceptibility, C. ***pneumoniae*** infections may relapse following antibiotic therapy

with these agents. In vitro studies on the persistence of Chlamydiae despite specific and. . .

SUMM . . . diagnosis of pathogenic infection as well as therapeutic approaches to manage the infection. Due to the highly infective nature of ***Chlamydia*** EBs and their ability to reinfect cells, there is also a need for antichlamydial therapy which totally eradicates this pathogen,. . .

SUMM [0008] The present invention provides a unique approach for the diagnosis and management of infection by ***Chlamydia*** species, particularly C. ***pneumoniae*** . The invention is based upon the discovery that a combination of agents directed toward many of the various stages of. . . ultimately prevent reinfection/reactivation of the pathogen. Accordingly, one embodiment of the invention pertains to methods of treating infection by a ***Chlamydia*** species, comprising administering to an individual in need thereof a combination of antichlamydial agents, comprising at least two agents, each. . .

SUMM . . . Use of the combination of antichlamydial agents or compositions thereof for the manufacture of a medicament for the management of ***Chlamydia*** infection is also described. In a particular embodiment, the agents can be assembled individually, admixed or instructionally assembled. The invention. . .

SUMM . . . during a course of therapy, the invention provides a means for packaging therapeutic agents, described herein, for the management of ***Chlamydia*** infection. For example, a pack can comprise at least two different agents, each of which is targeted against a different. . .

SUMM . . . the infection status of an individual and/or the progress of therapy in an individual undergoing therapy for infection caused by ***Chlamydia*** . The method comprises quantifying antibody titer or other measure to the pathogen and comparing the measure to antibody measure quantified. . . of the therapy. The invention also pertains to a method for monitoring the course of therapy for treating infection by ***Chlamydia*** , ,comprising determining presence or absence of ***Chlamydia*** in an infected individual at time intervals during course of therapy. In a particular embodiment, this is determined by PCR. . .

SUMM [0012] Detection of the presence of ***Chlamydia*** in a sample of biological material taken from an individual thought to be infected therewith is important in determining the. . . therapy and the agents to be used. This can be achieved by detecting the presence of DNA encoding MOMP of ***Chlamydia*** or other chlamydial genes in the individual. In one aspect of the invention, diseases associated with ***Chlamydia*** infection, such as inflammatory diseases, autoimmune diseases and diseases in which the individual is immunocompromised, can be treated by managing (i.e., significantly reducing infection or eradicating) the ***Chlamydia*** infection using the novel approach described herein. Both clinical and serological improvements/ resolutions in patient status have been demonstrated. The. . . agent(s) capable of significantly reducing/eliminating chlamydial infection. The method comprises preparing tissue culture from cell lines; inoculating these cells with ***Chlamydia*** in the absence of cycloheximide; allowing the ***Chlamydia*** to infect these cells for several days;

SUMM . . . suitable nucleotide amplification assay, such as PCR. Preferably the presence or absence of signal for amplified DNA encoding

MOMP of ***Chlamydia*** or other chlamydial protein is determined. Absence of a signal indicates a reduction in the degree of infection below that. . . are particularly useful as a drug screening tool for assessing the activity of single agents or combinations of agents against ***Chlamydia*** infection.

SUMM [0015] In one embodiment, a suitable nucleotide assay for identifying agents effective against a cryptic form of ***chlamydia*** comprises, in the presence of agent(s) to be tested, is performed by subjecting cultured cells to protease/reducing agent (e.g., dithiotreitol. . .

SUMM [0016] exposing DNA to appropriate polymerase, dNTPs and primers for DNA amplification of MOMP or other protein of the ***Chlamydia*** species; and determining the presence or absence of amplified DNA by visualizing the ethidium bromide treated DNA product by gel electrophoresis, for example. In particular embodiments, the ***Chlamydia*** species is C. ***pneumoniae*** and the appropriate primers are CHLMOMPDB2 and CHLMOMPCB2.

SUMM [0017] The invention further relates to a method of identifying cells containing a cryptic form of a ***Chlamydia*** species by a nucleic acid amplification technique (e.g., PCR) comprising subjecting cultured cells to protease digestion; stopping protease activity;

SUMM . . . heat-stable DNA polymerase, dNTPs and labeled primers (e.g., 3'-biotin labeled, 5'-biotin labeled) for amplification of DNA encoding MOMP of the ***Chlamydia*** species; washing the cells; exposing the cells to a reporter molecule (e.g., strepavidin-conjugated signal enzyme); exposing the cells to an. . .

SUMM [0019] A method of identifying cells containing a cryptic form of ***Chlamydia*** comprises treating cultured cells, thought to be infected with ***Chlamydia*** , with a disulfide reducing agent; subjecting cultured cells to protease digestion; exposing cells to appropriate polymerase, dNTPs and primers for. . . exposing the cells to an appropriate substrate for the reporter enzyme; and determining the presence of a cryptic form of ***Chlamydia*** by visualizing the amplified DNA encoding a chlamydial protein. Preferably the amplification technique is PCR and the primers are CHLMOMPDB2 and CHLMOMPCB2 of ***Chlamydia*** ***pneumoniae*** .

SUMM . . . similar method can be used as an assay for identifying an agent which is effective against a cryptic form of ***Chlamydia*** . Accordingly, the method comprises treating cultured cells grown in the absence of cycloheximide, thought to be infected with ***Chlamydia*** , with a disulfide reducing agent; allowing the ***chlamydia*** to replicate; adding a test agent; subjecting cultured cells to protease digestion; exposing cells to appropriate polymerase, dNTPs and primers. . . exposing the cells to an appropriate substrate for the reporter enzyme; and determining the presence of a cryptic form of ***Chlamydia*** by visualizing the amplified DNA encoding a chlamydial protein, such as MOMP.

SUMM [0022] The present invention pertains to methods for clearing biological material infected with ***Chlamydia*** to produce ***Chlamydia*** -free cell lines and animals, and to methods of maintaining biological material, e.g, cell lines and animals, such that they remain ***Chlamydia*** -free. According to the method, a biological material is cleared from ***Chlamydia*** infection by contacting the biological material with at least two agents but preferably three agents, each of which is targeted against a different phase of the

chlamydial life cycle, until the biological material no longer tests positive for ***Chlamydia*** .

SUMM [0024] Biological material that has been cleared of ***Chlamydia*** infection, according to the methods of this invention, are also described. The biological material can be a continuous cell line. . .

SUMM [0025] wherein "CF" is a shorthand annotation for " ***Chlamydia*** -free". Alternatively, the biological material can be an animal, such as a mouse, rabbit or other animal model, which is negative for ***Chlamydia*** .

SUMM [0026] The invention also pertains to methods of maintaining a ***Chlamydia*** -free status in animals and cell lines which have been cleared of ***Chlamydia*** infection by the methods of this invention, or have never been infected, such as their ***Chlamydia*** -free offspring or progeny. Cells or animals can be maintained as ***Chlamydia*** -free by maintaining them on antibiotics and/or treating their nutrients and environment to ensure that they are ***Chlamydia*** -free. Particularly, a source of nutrients to be administered to ***Chlamydia*** -free cells or animals can be treated to inactivate or remove any chlamydial elementary bodies therefrom. This can be accomplished by. . .

SUMM . . . pertains to a diagnostic kit or pack comprising an assembly of materials selected from the group consisting of antibiotics, reagents, ***Chlamydia*** -free cell lines, and combinations thereof, or other materials that would be necessary to perform any of the methods described herein.

SUMM [0028] The invention further relates to a method of detecting viable ***Chlamydia*** in a biological material suspected of being contaminated therewith, comprising culturing ***Chlamydia*** -free cells or animals in the presence of biological material and then determining the presence or absence of viable ***Chlamydia*** in the culture.

SUMM [0029] The invention also pertains to a method for differentiating porphyria caused by ***Chlamydia*** species from porphyria caused by a genetic disorder. The method comprises measuring peripheral red blood cell enzymes and/or performing a. . . more components of the heme pathway, the porphyria is not caused by a genetic disorder and may be caused by ***Chlamydia*** . The invention relates to a method for diagnosing secondary porphyria caused by ***Chlamydia*** in an individual having symptoms associated therewith, comprising determining the presence or amount of obligatory enzymes in heme biosynthesis in red blood cells of the individual and determining the presence of ***Chlamydia*** in the individual. The invention further relates to a method for differentiating secondary porphyria caused by ***Chlamydia*** from that caused by a genetic disorder in an individual, comprising treating infection by ***Chlamydia*** at many stages of its life cycle and then assessing whether porphyrins have been reduced, wherein a decrease in the porphyrin levels is indicative that the porphyria is secondary and caused by ***Chlamydia*** .

SUMM [0030] The subject invention also pertains to a method for treating porphyria caused by ***Chlamydia*** in an individual in need thereof, comprising reducing the levels of active stage, latent stage and elementary bodies of the. . .

SUMM . . . can be automated using a computerized system, for example, to formulate a drug therapy for management of infection caused by ***Chlamydia*** . The method comprises determining targets within the

chlamydial life cycle, for each determined target; identifying agents that are active against. . . combining at least a subset of the identified agents to provide a combination therapy for management of infection caused by ***Chlamydia*** , the agents in said subset individually being active against different targets in the life cycle of ***Chlamydia*** . The targets include identifying phases of the chlamydial life cycle and for each identified life cycle phase, determining at least. . .

DRWD [0034] FIGS. 1A and 1B show a sequence alignment of various ***Chlamydia*** MOMPs.

DRWD [0036] FIG. 3 illustrates the constant and variable domain (VD) of various ***Chlamydia*** species.

DETD . . . or in combination to eliminate or interfere with more than one of the distinct phases of the life cycle of ***Chlamydia*** species. These chlamydial phases include the intracellular metabolizing/replicating phase; the intracellular "cryptic" phases; and the extracellular EB phase. Current concepts. . .

DETD [0040] Diagnostic and therapeutic methods for the management of ***Chlamydia*** infections are described in detail below. For the purposes of this invention, "management of ***Chlamydia*** infection" is defined as a substantial reduction in the presence of all phases/forms of ***Chlamydia*** in the infected host by treating the host in such a way as to minimize the sequellae of the infection. ***Chlamydia*** infections can thus be managed by a unique approach referred to herein as "combination therapy" which is defined for the. . . or manage chlamydial infection. The diagnostic methods and combination therapies described below are generally applicable for infection caused by any ***Chlamydia*** species, including but not limited to C. ***pneumoniae*** , C. ***trachomatis*** , C. psittaci and C. pecorum. Infections in which the causative agent is C. ***pneumoniae*** are emphasized.

DETD [0041] Antichlamydial agents, which have been identified as effective against ***Chlamydia*** by the susceptibility testing methods described herein, can be used singly to affect ***Chlamydia*** in a single stage of its life cycle or as part of a combination therapy to manage ***Chlamydia*** infection. For example, compounds identified as anti-cryptic phase drugs, anti-EB phase drugs, anti-DNA-dependent RNA polymerase drugs and nicotinic acid cogener. . .

DETD [0042] Diagnosis of ***Chlamydia*** Infection

DETD [0043] The invention pertains to methods for diagnosing the presence of ***Chlamydia*** in a biological material, as well as to the use of these methods to evaluate the serological status of an. . .

DETD . . . more immunoglobulins, such as IgG, IgM, IgA and IgE, against antigenic determinants within the full length recombinant MOMPs of various ***Chlamydia*** species. Detection of IgG and/or IgM against antigenic determinants within the full length recombinant MOMP of C. ***pneumoniae*** is preferred. IgA determinations are useful in the analysis of humoral responses to ***Chlamydia*** in secretions from mucosal surfaces (e.g., lung, GI tract, gerontourinary tract, etc.). Similarly, IgE determinations are useful in the analysis of allergic [manifestatins] manifestations of disease. Table 1 below provides the GenBank Accession numbers of various MOMPs for ***Chlamydia*** species.

TABLE 1

Species	Strain	ID	GenBank Accession No.
C. ***trachomatis***		A	CTL/A M33636
C. ***trachomatis***		A	CTL/A M58938 M33535
C. ***trachomatis***		A	CTL/A J03813
C. ***trachomatis***		B	CTL/B M33636
C. ***trachomatis***		C	CTL/L M17343 M19128
C. ***trachomatis***		D	CTL/D A27838
C. ***trachomatis***		E	CTL/E X52557
C. ***trachomatis***		F	CTL/F X52080 M30501
C. ***trachomatis***		H	CTL/H X16007
C. ***trachomatis***		L1	CTL/L1 M36533
C. ***trachomatis***		L2	CTL/L2 M14738 M19126
C. ***trachomatis***		L3	CTL/L3 X55700
C. ***trachomatis***		Mouse Pneumo	CTL/MP X60678
C. pecorum	Ovine Polylarthritis	CPC/OP	Z18756
C. psittaci	Strain 6BC	CPS/6B	X56980
C. psittaci	Feline	CPS/F	X61096
C. ***trachomatis***		Da	CTL/DA X62921 S45921
C. ***pneumoniae***		TWAR	CPN/HU1 M64064 M34922 M64063
C. ***pneumoniae*** (? C. pecorum)		Horse	CPN/EQ2 L04982
C. ***pneumoniae***		TWAR	CPN/MS not assigned
C. Psittaci	Horse	CPS/EQ1	L04982

DETD . . . with no cross reactivity to other immunoglobulins (Pharmagen; Clone G20-127, Catalog No. 34152D). Peptide-based immunoassays can be developed which are ***Chlamydia*** specific or provide species specificity, but not necessarily strain specificity within a species, using monoclonal or polyclonal antibodies that are. . .

DETD [0047] Recombinant-based immunological assays have been developed to quantitate the presence of immunoglobulins against the ***Chlamydia*** species. Full length recombinant ***Chlamydia*** MOMP can be synthesized using an appropriate expression system, such as in E. coli or Baculovirus. The expressed protein thus. . . for suitable immunological methods, as discussed above. Protein-based immunological techniques can be designed that are species- and strain-specific for various ***Chlamydia*** .

DETD [0048] Diagnosis of chlamydial infection can now be made with an improved IgM/IgG C. ***pneumoniae*** method of quantitation using ELISA techniques, Western blot confirmation of ELISA specificity and the detection of the MOMP gene of C. ***pneumoniae*** in serum using specific amplification primers that allow isolation of the entire gene for analysis of expected strain-specific differences.

DETD . . . (PCR) methodologies which comprise solution PCR and in situ PCR, to detect the presence or absence of unique genes of

Chlamydia . Species-specific assays for detecting ***Chlamydia*** can be designed based upon the primers selected. Examples of suitable PCR amplification primers are illustrated below in Table 2.. . . CTL/L2 ATGAAAAAACTCTTGAAATCGGTATTAGTGTTTGCCGCTTTGA GTTCTGC 17

X55700 CTL/L3 ATGAAAAAACTCTTGAAATCGGTATTAGTGTTTGCCG CTTTGAGTTCTGC 18

X60678 CTL/MP ATGAAAAAACTCTTGAAATCGGTATTAGCATTTGCCG TTTTGGGTTCTGC 19

Chlamydial Species	Strain	SEQ ID NO.	Terminal Fifty Nucleotides
C. ***pneumoniae***	TWAR	CPNHU1	GTTTAATTAACGAGAGAGCTGCTCACGTATCTGGTCAGTTCAGATTCTAA 20
C. ***pneumoniae***	MS	CPNHU2	GTTTAATTAACGAGAGAGCTGCTCACGTATCTGGTCAGTTCAGATTCTAA 21
C. psittaci	Horse	CPNEQ1	CAACGTTAATCGACGCTGACAAATGGTCAATCA CTGGTGAAGCACGCTTA 22
C. ***pneumoniae***	Horse	CPNEQ2	GTTTAATTAACGAGAGAGCTGCTCACATATCTGGTCAGTTCAGATTCTAA 23
C. psittaci	SBE	CPS/6B	AACGTTAATCGACGCTGACAAATGGTCAATCAC TGGTGAAGCACGCTTAA 24
C. psittaci	Ewe abortion	CPS/AB1	AACGTTAATCGACGCTGACAAATGGTCAATCAC TGGTGAAGCACGCTTAA 25
C. psittaci	Bovine abortion	CPS/AB2	GCTTAATCAATGAAAGAGCCGCTCACATGAATG CTCAATTCAGATTCTAA 26
C. psittaci	Avian	CPS/AV/C	GCTTAATCAATGAAAGAGCTGCTCACATGAATG CTCAATTCAGATTCTAA 27
C. psittaci	Feline	CPS/F	GCTTAATCGACGAAAGAGCTGCTCACATTAATG CTCAATTCAGATTCTAA 28
C. ***trachomatis***	Hu/A	CTL/A	CGCAGTTACAGTTGAGACTCGCTTGATCGATGAGAGAGCAGCTCACGTAA 29

C. ***trachomatis*** Hu/C CTL/C
GCTTGATCGATGAGAGAGCAGGTCACGTAAATGCACAATTCCGGTTCTAA
30

C. ***trachomatis*** Hu/Da CTL/DA
GCTTGATCGATGAGAGAGCAGCTCACGTAAATGCACAATTCCGCTTCTAA
31

C. ***trachomatis*** HU/E CTL/E
CGCTTGATCGATGAGAGACTGCTCACGTAAATGCACAATTCCGCTTCTAA
32

C. ***trachomatis*** Hu/F CTL/F
GCTTGATCGATGAGAGAGCTGCTCACGTAAATGCACAATTCCGCTTCTAA
33

C. ***trachomatis*** Hu/H CTL/H
GCTTGATCGATGAGAGAGCAGCTCACGTAAATGCACAATTCCGCTTCTAA
34

C. ***trachomatis*** Hu/L1 CTL/L1
GCTTGATCGATGAGAGAGCTGCTCACGTAAATGCACAATTCCGCTTCTAA
35

C. ***trachomatis*** Hu/L2 CTL/L2
GCTTGATCGATGAGAGAGCTGCTCACGTAAATGCACAATTCCGCTTCTAA
36

C. ***trachomatis*** Hu/L3 CTL/L3
GCTTGATCGATGAGAGAGCAGCTCACGTAAATGCACAATTCCGCTTCTAA
37

C. ***trachomatis*** Mouse CTL/MP
GCTTGATCGATGAAAGAGCAGCTCACGTAAATGCTCAGTTCCGTTTCTAA
38

.sup.aSequence from a cerebral spinal fluid of a patient with multiple sclerosis isolated by the inventors. Sequence is identical to TWAR C. ***pneumoniae*** with exception of a C/T mutation at NT 54 and a G/A mutation at NT 126.

.sup.bTerminator condon underlined

DETD [0050]

TABLE 3

Primers for PCR Amplification of Entire MOMP Gene.sup.a

Chlamydia Species	Strain	SEQ ID NO.	Plus Strand Primer Sequence
C. ***pneumoniae***	TWAR	CHLMOMP	ATGAAAAAAC TCTTAAAGTC

GGCGTTATTA 61.4.degree. 105
DB2 TCCGCCGC

C. ***trachomatis*** L2 CTMOMP ATGAAAAAAC TCTTGAAATC
GGTATTAGTG 61.2.degree. 106
L2DB TTTGCCGCTT TGAG

C. psittaci Feline PSOMP ATGAAAAAAC TCTTAAAATC GGCATTATTA
62.1.degree. 107
FPN-D TTTGCCGCTG CGGG

C. psittaci 6BC PSOMP ATGAAAAAAC TCTTGAAATC GGCATTATTG
63.0.degree. 108
6BC-b TTTGCCGCTA CGGG

C. ***trachomatis*** Mouse CTMU ATGAAAAAAC TCTTGAAATC
GGTATTAGCA 63.5.degree. 109
MOMP-D TTTGCCGTTT TGGGTTCTGC

SEQ
Chlamydia Minus Strand Primer
ID
Species Strain ID Sequence
T.sub.m.sup.b NO.

C. ***pneumoniae*** TWAR CHLMOMP TTAGAATCTG AACTGACCAG
ATACGTGAGC 64.4.degree. 110
CB2 AGCTCTCTCG

C. trachomamis L2 CWOMP TTAGAAGCGG AATTGTGCAT TTACGTGAGC
61.5.degree. 111
L2CB AGCTC
. . . TAATGTGAGC 62.2.degree. 112
FPN_C AGCTCTTTCG TCG

C. psittaci 6BC PSOMP TTAGAATCTG AATTGACCAT TCATGTGAGC
63.4.degree. 113
GBC_C AGCTCTTTCA TTGATTAAGC G

C. ***trachomatis*** Mouse CTMU TTAGAAACGG AACTGAGCAT
TTACGTGAGC 63.2.degree. 114
MOMP_C TGCTCTTTCA TC

.sup.aAll primers amplify under identical amplication conditions: 94.degree. C.
for 1. . .
DETD . . . clinical management of the chlamydial infection. Serological improvement can be based upon the current serological criteria for eradication of chronic ***Chlamydia*** reported below in Table 4.
TABLE 4

Serological Criteria for Eradication
of Chronic ***Chlamydia*** ***pneumoniae*** Infection

IgM .ltoreq.1:25
IgG Stable titer 1:100
PCR Negative

DETD . . . bromide staining and UV light detection. PCR primers can be designed to selectively amplify DNA encoding MOMP of a particular ***Chlamydia*** species, such as the MOMP of C. ***pneumoniae*** , C. pecorum, C. ***trachomatis*** , C. psittaci (See FIG. 1). Primers that are from about 15-mer to about 40-mer can be designed for this purpose.

DETD [0058] Clearing and Maintaining ***Chlamydia*** -Free Organisms

DETD [0059] The present invention provides a unique approach for creating and maintaining animals and cell lines which are free of ***Chlamydia*** infection. Also described herein are methods for creating nutrients and culture media that are suitable for use with animals and cell lines that have been cleared of ***Chlamydia*** infection.

DETD [0060] Attempts to culture isolates of C. ***pneumoniae*** from blood and cerebrospinal fluid (CSF) have resulted in the discovery that the continuous cell lines routinely used to cultivate C. ***pneumoniae*** are cryptically infected with C. ***pneumoniae*** . These include not only in house stocks of HeLa, HL, H-292, HuEVEC and McCoy cells, but also stocks obtained from. . . for HL cells, as well as a commercial supplier (Bartells) of H-292 and McCoy cells for the clinical culture of ***Chlamydia*** . The presence of a cryptic form of C. ***pneumoniae*** in these cells has been repeatedly demonstrated by solution PCR amplifying the MOMP. In situ PCR in HeLa cells against. . . be present in 100% of cells. Nevertheless, fluoroscenated mAb to LPS in McCoy cells does not yield any indication of ***Chlamydia*** (i.e., reactive against all ***Chlamydia***) while fluoroscenated mAb to C. ***pneumoniae*** MOMP yields a generalized fluorescence throughout the cytoplasm that can be confused with non-specific autofluorescence. Infection with ***Chlamydia*** ***trachomatis*** (Bartells supply) yields the typical inclusion body staining with the LPS mAb (i.e., cross reactive with all species of ***Chlamydia***) with no change in cytoplasmic signal with anti-MOMP mAb against C. ***pneumoniae*** . These findings (solution PCR, in situ PCR, mAb reactivity) were interpreted as consistent with a cryptic (non-replicating) infection by C. ***pneumoniae*** of cells commonly used to culture the organism. Further, virtually all untreated rabbits and mice tested to date have PCR signals for the C. ***pneumoniae*** MOMP gene.

DETD [0061] This creates a currently unrecognized problem of major significance for those clinical labs providing C. ***pneumoniae*** culture services as well as investigators who now do not know whether their results in animals or in cell culture. . . by cryptic chlamydial contamination. Clinical and research laboratories currently have no way to determine whether an organism is, in fact, ***Chlamydia*** -free.

DETD [0062] This invention pertains to a method for clearing cells and animals of C. ***pneumoniae*** and keeping them clear. Clearing them entails contacting the infected organism with agents used singly or in combination to eliminate or interfere with more than one of the distinct phases of the life cycle of ***Chlamydia*** species. Keeping them clear entails either maintaining them on antibiotics and/or treating their nutrients and environment to ensure they are ***Chlamydia*** -free. In a preferred embodiment, maintenance conditions comprise a

combination of isoniazid (INH) (1 .mu.g/ml), metronidazole (1 .mu.g/ml), and dithiothreitol (10. . .

DETD [0063] These techniques have now made it possible to create a variety of ***Chlamydia*** -free (CF) organisms, including continuous cell lines called HeLa-CF, HL-CF, H-292-CF, HuEVEC-CF, McCoy-CF, African green monkey and other cell lines that. . .

DETD [0064] Because ***Chlamydia*** is highly infectious, organisms which have been cleared of extracellular, replicating and cryptic infections must be protected from exposure to. . . have discovered that many of the nutrients and other materials used to maintain continuous cell lines are contaminated with viable ***Chlamydia*** EBs. For example, every lot of fetal calf serum has tested positive for the ***Chlamydia*** MOMP gene by PCR. Since extensive digestion is required for isolation of the DNA, we have concluded it is bound in EBs. C. ***pneumoniae*** can also be cultured directly from fetal calf serum. Thus, it is necessary to inactivate EBs in these materials, such as culture media and nutrients, used to maintain the ***Chlamydia*** -free status of the organism. Collectively these materials are referred to herein as "maintenance materials".

DETD [0065] In one embodiment, nutrients and culture media are subjected to gamma irradiation to inactivate ***Chlamydia*** therein. Preferably, the material should be irradiated for a period of time sufficient to expose the material to at least. . . reducing agent, preferably dithiothreitol (e.g., about 10 .mu.M concentration), before the materials are passed through a filtration system to remove ***Chlamydia*** therefrom.

DETD [0066] In order to insure that research tools, such as cell lines and animals, remain ***Chlamydia*** -free, an assay has been designed to evaluate whether an organism is ***Chlamydia*** -free. The method comprises obtaining a sample of cells or tissue culture;

DETD [0068] determining the presence or absence of ***Chlamydia*** nucleic acid by a suitable amplification technique, such as PCR. The absence of nucleic acid amplification signal is indicative that the status of the organism is ***Chlamydia*** -free.

DETD [0069] [Susceptability] Susceptibility Testing for Evaluating Active Agents Against Various Forms of ***Chlamydia***

DETD [0070] This invention pertains to novel approaches for the susceptibility testing of ***Chlamydia*** species that are necessitated by the complex life cycle of the chlamydial pathogen as well as by its diverse, extensive,. . .

DETD . . . to successfully and totally eradicate chronic chlamydial infections. This is because the current susceptibility testing methods measure only replication of ***chlamydia*** and ignores the well-known "cryptic phase" in which intracellular Chlamydiae are not actively replicating. Moreover, it has also been discovered. . .

DETD . . . the invention pertains to methods for evaluating the susceptibility of the distinct phases and stages of the life cycle of ***Chlamydia*** , particularly the cryptic phase to a particular agent(s), since prior techniques have failed, heretofore, to appreciate the need for drugs that can clear infected cells of cryptic ***Chlamydia*** . A preferred drug screening method which accomplished this objective utilizes tissue culture cells which are maintained, in the absence of. . .

DETD [0075] Cryptic infection is uncommon in cells used in standard cell culture susceptibility techniques because ***Chlamydia*** in

cycloheximide-paralyzed cells need not compete with the host cell for metabolites and hence are encouraged to replicate. The in. . . or combination of compounds to be evaluated as an antichlamydial agent for its ability to significantly reduce the presence of ***Chlamydia*** in living cells. For example, a test agent can include, but is not limited to, antibiotics, antimicrobial agents, antiparasitic agents,. .

.

DETD . . . as PCR) are used to ascertain the presence or absence of signal for chlamydial DNA encoding MOMP or another unique ***Chlamydia*** gene to determine whether the test agent or combination of agents is/are effective in reducing ***Chlamydia*** infection. The loss of signal (i.e., below the detectable level of the nucleic acid amplification technique) in cells with antibiotic(s) versus its presence in controls is an indication of efficacy of the agent or combination of agents against ***Chlamydia*** .

DETD . . . of this invention can be used to identify an agent or agents which are targeted against any particular species of ***Chlamydia*** and can be used to identify agent(s) targeted against the cryptic form of the pathogen, i.e., is capable of inhibiting. . . embodiment, this is done by performing the susceptibility test while placing the cells under stringent environmental conditions known to induce ***Chlamydia*** to enter a cryptic phase. Agents that are effective against ***Chlamydia*** , as ascertained by the susceptibility testing protocols described herein, can be used as part of a therapy for the management of ***Chlamydia*** infections. Suitable therapeutic protocols are described in detail below, with a particular focus on targeting agents toward specific stages of. . .

DETD [0080] In one embodiment, a suitable nucleic acid assay for identifying agents effective against the cryptic form of ***Chlamydia*** comprises, in the presence of agent(s) to be tested, subjecting cultured cells to reducing agent (e.g., dithiotreitol) and protease digestion. . . treated solution; exposing DNA to appropriate polymerase, dNTPs and primers for DNA amplification of MOMP or other protein of the ***Chlamydia*** species; and determining the presence or absence of amplified DNA by visualizing the ethidium bromide treated DNA product by gel electrophoresis, for example, or alternatively by Southern Blot. In particular embodiments, the ***Chlamydia*** species is C. ***pneumoniae*** and the appropriate primers are CHLMOMPDB2 and CHLMOMPCB2.

DETD [0081] The invention further relates to a method of identifying cells containing a non-EB cryptic form of a ***Chlamydia*** species by a nucleic acid amplification technique (e.g., PCR) comprising subjecting cultured cells to protease digestion; stopping protease activity;

DETD . . . heat-stable DNA polymerase, dNTPs and labeled primers (e.g., 3'-biotin labeled, 5'-biotin labeled) for amplification of DNA encoding MOMP of the ***Chlamydia*** species; washing the cells; exposing the cells to a reporter molecule (e.g., strepavidin-conjugated signal enzyme); exposing the cells to an. . .

DETD [0083] The invention pertains to a method of identifying cells containing a cryptic form of ***Chlamydia*** . The method comprises treating cultured cells, thought to be infected with ***Chlamydia*** , with a disulfide reducing agent; subjecting cultured cells to protease digestion; exposing cells to appropriate polymerase, dNTPs and primers for. . . exposing the cells to an appropriate substrate for the reporter enzyme; and determining the presence of a cryptic form of

Chlamydia ,by visualizing the amplified DNA encoding a chlamydial protein. Preferably, the amplification technique is PCR and the primers are CHLMOMPDB2 and CHLMOMPCB2 of ***Chlamydia*** pneumonlae.

DETD . . . similar method can be used as an assay for identifying an agent which is effective against a cryptic form of ***Chlamydia*** . Accordingly, the method comprises treating cultured cells grown in the absence of cycloheximide; thought to be infected with ***Chlamydia*** , with a disulfide reducing agent; allowing the ***Chlamydia*** to replicate; adding a test agent; subjecting cultured cells to protease digestion; exposing cells to appropriate polymerase, dNTPs and primers. . . enzyme; exposing the cells to an appropriate substrate for the reporter enzyme; and determining the presence of cryptic form of ***Chlamydia*** by visualizing the amplified DNA encoding a chlamydial protein, such as MOMP.

DETD . . . susceptibility test can be used to evaluate the status of a human or animal undergoing therapy for the management of ***Chlamydia*** infection. For example, a biological material is isolated from the human or animal to undergo combination therapy. The biological material is treated such that the ***Chlamydia*** is isolated therefrom. This chlamydial isolate is allowed to infect ***Chlamydia*** free cells.

DETD . . . method has revealed, for example, that antimicrobial therapy with the triple agents, INH, metronidazole and penicillamine, can completely eradicate C. ***pneumoniae*** from infected mice in four months. Moreover, following complete eradication of chlamydiae, multiple attempts to reinfect these cured mice via. . .

DETD . . . of determining the presence of cryptic chlamydial infections in an animal or cell culture is to expose the culture to ***chlamydia*** -stimulating compounds. Such compounds include (but are not limited to) cycloheximide, corticosteriods (such as prednisone) and other compounds which are known. . .

DETD [0091] Antichlamydial Therapy Directed Toward the Initial Stage of ***Chlamydia*** Infection

DETD . . . and electron transfer proteins, as well as nitroreductases. Based upon this, it has been discovered that the initial phase of ***Chlamydia*** infection is susceptible to the antimicrobial effects of nitroimidazoles, nitrofurans and other agents directed against anaerobic metabolism in bacteria.

DETD . . . including ribosomes, DNA and RNA. Nitroimidazoles and nitrofurans currently are not considered to possess antimicrobial activity against members of the ***Chlamydia*** species. This lack of antimicrobial activity, however, is due to the fact that conventional susceptibility testing methods only test for effect on the replicating form of ***Chlamydia*** species.

DETD . . . an agent, such that the modification results in an agent having similar or increased, but not significantly decreased, effectiveness against ***Chlamydia*** , compared to the effectiveness of the parent agent from which the analog or derivative is obtained. This comparison can be. . .

DETD [0097] Novel Antichlamydial Therapy Directed Toward the Replicating and Cryptic Stationary Phases of ***Chlamydia*** Infection

DETD [0098] A unique class of antichlamydial agents that is effective against the replicating and cryptic stationary phases of ***Chlamydia*** (and possibly against some other stages of the cryptic phase) have been

identified using the susceptibility tests described herein. This. . . [susceptability] susceptibility testing methodologies, it has been discovered that these agents, in combination with other antibiotics, are particularly effective against ***Chlamydia*** . It is believed that the isonicotinic acid congeners target the constitutive production of catalase and peroxidase, which is a characteristic of microorganisms, such as mycobacteria, that infect monocytes and macrophages. ***Chlamydia*** can also successfully infect monocytes and macrophages.

DETD [0099] Using INH to eradicate ***Chlamydia*** from macrophages and monocytes subsequently assists these cells in their role of fighting infection. However, these agents appear to be. . .

DETD . . . and its congeners can be used to clear infection from monocytes and/or macrophages. When monocytes and macrophages are infected by ***Chlamydia*** , they become debilitated and cannot properly or effectively fight infection. It is believed that, if the chlamydial infection, per se,. . . one aspect of the invention provides a specific method for reempowering monocytes or macrophages that have been compromised by a ***Chlamydia*** infection and, in turn, comprise treating the infection in other sites. Such compromised macrophages or monocytes can be activated by. . .

DETD [0101] Therapy Directed Toward Elementary Bodies of ***Chlamydia***

DETD . . . discovered that adverse conditions, such as limited nutrients, antimicrobial agents, and the host immune response, produce a stringent response in ***Chlamydia*** . Such adverse conditions are known to induce stringent responses in other microorganisms (C. W. Stratton, In: Antibiotics in Laboratory Medicine,. . . Fourth Edition. Lorian V (ed) Williams & Wilkins, Baltimore, pp 579-603 (1996)) and not surprisingly induce a stringent response in ***Chlamydia*** . This stringent response in ***Chlamydia*** alters the morphological state of the intracellular microorganism and creates dormant forms, including the intracellular EB, which then can cryptically. . . the extracellular milieu. Thus, it is necessary to utilize a combination of agents directed toward the various life stages of ***Chlamydia*** and, in particular, against the elementary body for successful management of infection.

DETD . . . also believed that [persistance] persistence of chlamydial infections, in part, may be due to the presence of cryptic forms of ***Chlamydia*** within the cells. This cryptic intracellular chlamydial form apparently can be activated by certain host factors such as cortisone (Yang. . . Infection and Immunity, 39:655-658 (1983); and Malinverni et al., The Journal of Infectious Diseases, 172:593-594 (1995)). Antichlamydial therapy for chronic ***Chlamydia*** infections must be continued until any intracellular EBs or other intracellular cryptic forms have been activated and extracellular EBs have. . . their respective hosts by reducing disulfide bonds which maintain the integrity of the outer membrane proteins of the EBs. For ***Chlamydia*** , disruption of the outer membrane proteins of EBs thereby initiates the transition of the EB form to the RB form.. . .

DETD [0106] Currently Recognized Agents Active Against ***Chlamydia*** Replication

DETD . . . they begin to utilize active transcription of chlamydial DNA and translation of the resulting mRNA. As such, these forms of ***Chlamydia*** are susceptible to currently used antimicrobial agents. The antichlamydial effectiveness of these agents can be

significantly improved by using them in combination with other agents directed at different stages of ***Chlamydia*** life cycle, as discussed herein.

DETD . . . as well as those which are preferred, are illustrated below in Table 5.

TABLE 5

Agents Effective Against the Replicating Phase of ***Chlamydia***

Drug Class	Examples	Preferred
Quinolones/ Fluoroquinolones	Ofloxacin	Levofloxacin
	Levofloxacin	
	Trovafloxacin	
	Sparfloxacin	
	Norfloxacin	
	Lomefloxacin	
	Cinoxacin	
	Enoxacin	
	Nalidixic Acid	
	Fleroxacin	
	Ciprofloxacin	
Sulfonamides. . .		

DETD [0109] All members of the ***Chlamydia*** species, including C. ***pneumoniae*** , are considered to be inhibited, and some killed, by the use of a single agent selected from currently used antimicrobial agents such as those described above. However, using the new susceptability test, the inventors have found complete eradication of ***Chlamydia*** cannot be achieved by the use of any one of these agents alone because none are efficacious against all phases of the ***Chlamydia*** life cycle and appear to induce a stringent response in ***Chlamydia*** causing the replicating phase to transform into cryptic forms. This results in a persistent infection in vivo or in vitro. . . DNA. Nevertheless, one or more of these currently used agents, or a new agent directed against the replicating phase of ***Chlamydia*** , should be included as one of the chlamydial agents in a combination therapy in order to slow or halt the. . .

DETD . . . attempting to manage or eradicate a systemic infection, it is critical to target multiple phases in the life cycle of ***Chlamydia*** , otherwise viable ***Chlamydia*** in the untargeted phases will remain after therapy and result in continued, chronic infection. This fundamental insight is at the. . .

DETD [0119] 2. Evaluate the relative importance of targeting each particular phase in eradicating reservoirs of ***Chlamydia*** from the host organism. For example, the life-cycle stages listed in step 1 can be prioritized based on the following. . .

DETD . . . cycle seen in cycloheximide-treated eukaryotic cells is an artifact of an a typical, cell culture environment designed primarily to propagate ***Chlamydia*** .

DETD [0122] c. The transition phases represent only a small portion of ***Chlamydia*** in chronic infections.

DETD [0123] 3. Identify "targets" for each phase of the selected life cycle phases. A target is an attribute of ***Chlamydia*** which is vulnerable during a particular life cycle phase. For example, the

disulfide bonds in MOMP are a target during. . .

DETD . . . of the chlamydial life cycle leads to a re-prioritization or even sub-division of the life-cycle phases, new theoretical targets within ***Chlamydia*** are identified, or new drugs are developed which attack currently known or new targets within ***Chlamydia*** . For example, the phases of the life cycle could be further sub-classified based on the type of host cell the. . . using Theorectical Effect on Various Targets within the Chlamydial Life Cycle to Pick a Combination Therapy

Potentially vulnerable attributes of ***Chlamydia*** :

Constitutive DNA-Ribosomes production of dependent involved in of Disulfide Non-oxidative peroxidases and RNA Folic acid protein bonds. . .

DETD [0140] An association has been discovered between chronic ***Chlamydia*** infection of body fluids and/or tissues with several disease syndromes of previously unknown etiology in humans which respond to unique. . . neural-mediated hypotension); Pyoderma Gangrenosum (PG), Chronic Fatigue (CF) and Chronic Fatigue Syndrome (CFS). Other diseases are under investigation. Correlation between ***Chlamydia*** infection and these diseases has only recently been established as a result of the diagnostic methodologies and combination therapies described. . .

DETD [0141] Based on this evidence, published evidence of an association between atherosclerosis and ***Chlamydia*** (Grupta et al., Circulation ,96:404-407,(1997)), and an understanding of the impact ***Chlamydia*** infections have on infected cells and the immune systems, the inventors have discovered a connection between ***Chlamydia*** and a broad set of inflammatory, autoimmune, and immune deficiency diseases. Thus, the invention describes methods for diagnosing and/or treating disease associated with ***Chlamydia*** infection, such as autoimmune diseases, inflammatory diseases and diseases that occur in immunocompromised individuals by diagnosing and/or treating the ***Chlamydia*** infection in an individual in need thereof, using any of the assays or therapies described herein. Progress of the treatment can be evaluated serologically, to determine the presence or absence of ***Chlamydia*** using for example the diagnostic methods provided herein, and this value can be compared to serological values taken earlier in. . . alternate compounds should be substituted in order to achieve the lower antibody titers that demonstrate specific [susceptability] susceptibility of the ***Chlamydia*** to the new regimen.

DETD [0142] A replacement or substitution of one agent with another agent that is effective against the same life stage of ***Chlamydia*** is desirable. The therapies described herein can thus be used for the treatment of acute and chronic immune and autoimmune diseases when patients are demonstrated to have a ***Chlamydia*** load by the diagnostic procedures described herein which diseases include, but are not limited to, chronic hepatitis, systemic lupus erythematosus,. . .

DETD . . . peripheral neuropathy, chronic or recurrent sore throat,

laryngitis, tracheobronchitis, chronic vascular headaches (including migraines, cluster headaches and tension headaches) and ***pneumonia*** when demonstrated to be pathogenically related to ***Chlamydia*** infection.

DETD [0145] Treatable disorders when associated with ***Chlamydia*** infection also include, but are not limited to, neurodegenerative diseases, including, but not limited to, demyelinating diseases, such as multiple. . .

DETD . . . the diseases indicated were observed and are reported in Example 5. The data provides evidence to establish that treatment of ***Chlamydia*** infection results in the serological and physical improvement of a disease state in the patient undergoing combination therapy. These observations. . .

DETD [0149] Other Diseases of Unknown Etiology with New Evidence for a ***Chlamydia*** ***Pneumoniae*** Etiology

DETD [0150] Both C. ***trachomatis*** and C. psittaci exhibit a protean disease complex dependent on different serovars. One known basis for this diversity to date is the amino acid sequence of the MOMP. FIG. 1 shows a sequence alignment of various ***Chlamydia*** MOMPs. Note that the size and sequence are relatively homologous except for the four variable regions that are responsible for the serovar (serotype) basis of classification. Further, it has been discovered that C. ***pneumoniae*** infects blood vessel endothelial cells from which EBs are released in the blood stream. In addition, macrophages are known targets for C. ***pneumoniae*** and may serve as reservoirs and provide an additional mechanism of transmission. C. ***pneumoniae*** is thus able to spread throughout the human body, establishing infection in multiple sites and in multiple organ systems. Infected. . .

DETD . . . intended to embrace both humans and animals. Virtually all rabbits and mice tested to date have PCR signals for C. ***pneumoniae*** . They can be used as appropriate animal models for treatment using specific combination antibiotics to improve therapy. (Banks et al.,. . .

DETD [0152] Coupled with these developments are the recently developed rabbit models of coronary artery disease, where rabbits exposed to C. ***pneumoniae*** subsequently develop arterial plaques similar to humans (Fong et al., J. Clin. Microbiol. 35:48-52 (1997)). Most recently, a study at. . . Hospital in London found that roughly {fraction (3/4)} of 213 heart attach victims have significant levels of antibodies to C. ***pneumoniae*** antibody and that those that have such antibodies achieve significantly lower rates of further adverse cardiac events when treated with. . .

DETD . . . also been introduced based on the report that Vitamin C (ascorbic acid) at moderate intracellular concentrations stimulates replication of C. ***trachomatis*** (Wang et al., J. Clin. Micro. 30:2551-2554 (1992)) as well as its potential effect on biofilm charge and infectivity of. . .

DETD [0159] ***Chlamydia*** is a parasite of normal energy production in infected eukaryotic cells. As a result, host cells have insufficient energy available. . . cell mitochondria to attempt to synthesize certain critical enzymes involved in energy production in order to increase energy production. Because ***Chlamydia*** also prevents this synthesis from completing, these enzyme's precursors, called porphyrins, build up in cell and often escape into the. . .

DETD . . . this secondary form of porphyria, a unique approach for the

diagnosis and treatment of obligatory and secondary disorders caused by ***Chlamydia*** infections has been developed. The adjunctive therapy described herein can be used in combination with the appropriate antimicrobial therapy required. . .

DETD . . . Y., Microbiological Reviews, 42:247-306 (1978); McClairty, G., Microbiology, 2:157-164(1994)). The transition of elementary bodies (EBs) to reticulate bodies (RBs) for ***Chlamydia*** species requires the presence of functioning mitochondria in the infected cell as well as the production by the host cell. . .

DETD [0174] B. ***Chlamydia*** and Secondary Porphyria

DETD . . . step in the biosynthesis of heme as it catalyses the oxidative entry of coproporphyrinogen into the mitochondria matrix as protoporphyrin; ***Chlamydia*** interfere with this step by reducing electron transfer in the host cell. When coproporhyrinogen is unable to return to the. . .

DETD [0176] Depletion of host cell energy by the intracellular infection with ***Chlamydia*** species causes additional energy-related complications. As fewer electrons are available to move through the electron transport chain of the host. . .

DETD . . . the classical manifestations of hereditary porphyria. As the chlamydial-infected host cells lyse, as happens in the normal life cycle of ***Chlamydia*** , the intracellular porphyrins are released and result in a secondary porphyria. Moreover, when the chlamydial infection involves hepatic cells, the. . . is a heme-based enzyme. Hence, the biosynthesis of heme in the liver becomes increased. When hepatic cells are infected with ***Chlamydia*** species, the decreased energy in the host cell does not allow heme biosynthesis to go to completion and porphyrins in the liver/entero-hepatic circulation are increased. It also has been noted that any host cell infected with ***Chlamydia*** species has an increased amount of intracellular porphyrins that are released when antimicrobial agents kill the microorganism.

DETD . . . clearly is of paramount importance in dealing with chronic systemic chlamydial infections as are seen with intravascular infections caused by ***Chlamydia*** ***pneumoniae*** .

DETD . . . (Kordac V., Neoplasma, 19:135-139 (1972); Lithner et al, Acta Medica Scandanavia, 215:271-274 (1984)). Of particular interest is that infection with ***Chlamydia*** ***pneumoniae*** has been associated with lung cancer (Cerutti P A., Science, 227:375-381 (1985)).

DETD . . . foregoing discussion of the etiology of porphyria, one aspect of the invention pertains to methods for differentiating porphyria caused by ***Chlamydia*** from that caused by a latent genetic disorder in an individual. The method comprises treating infection by ***Chlamydia*** at all stages of its life cycle, using the therapies described in detail elsewhere in this disclosure, and then assessing. . . symptoms of porphyria (e.g., biochemical, enzymatic or physical manifestation) are indicative that the porphyria is a secondary porphyria caused by ***Chlamydia*** .

DETD . . . is suggestive of a non-genetic porphyria, such as chlamydially induced secondary porphyria. For example, in one embodiment, porphyria caused by ***Chlamydia*** in an individual having symptoms associated therewith can be diagnosed by determining the presence and/or amount of obligatory enzymes in. . .

DETD [0184] As discussed above, some patients having a ***Chlamydia*** -induced porphyria do not have abnormal levels of heme precursors. For those patients it may be appropriate to determine the presence of

Chlamydia as well as porphyrins in the individual. The presence of both the pathogen and porphyrins (e.g., determined by ELISA assay. . .

DETD . . . well as Vitamin B12 (cobalamin), which is molecularly similar to these metabolites, in patients with active systemic infection with C. ***pneumoniae*** . The antibodies are primarily IgM; this is similar to the antibody responses to the MOMP of C. ***pneumoniae*** in severely symptomatic patients. Example 8 illustrates titers in symptomatic patients with systemic C. ***pneumoniae*** infections. The presence of antibodies to Vitamin B12 may have functional significance by decreasing the amount of bioavilable Vitamin B12. Thus, a ***Chlamydia*** infection may cause a previously unrecognized secondary Vitamin B12 deficiency. Administration (e.g., intramuscular) of large quantities of VitaminB12 (1000 to. . .

DETD [0188] Treatment of ***Chlamydia*** infection may [exaserbate] exacerbate secondary porphyria by increasing the metabolism of cryptic ***Chlamydia*** or by accelerating the death of infected cells with elevated intracellular porphyrin levels.

DETD [0205] To reduce severe porphyric attacks during therapy for chronic ***Chlamydia*** infections, the use of hemodialysis, plasmapheresis, chelating agents and/or intravenous hematin may be needed. Any one of these or a. . .

DETD . . . nutritional formulations including beverages and foods such as nutritional bar, for the management of non-genetic, secondary porphyria caused by a ***Chlamydia*** infection.

DETD . . . to ameliorate conditions/symptoms associated with the disease states described above, when the disease is onset or aggravated by infection by ***Chlamydia*** . The agents of this invention can be administered to animals including, but not limited to, fish, amphibians, reptiles, avians and. . .

DETD . . . thereof. The agents can also be used for the manufacture of a medicament for therapy of a disease associated with ***chlamydia*** infection, such as autoimmune disease, inflammatory disease, immunodeficiency disease.

DETD [0235] Polymerase Chain Reaction (PCR) for the Full Length MOMP Gene of C. ***Pneumoniae*** and Other Species of ***Chlamydia*** (Diagnostic)

DETD . . . #1154, which on repeated assay without reducing agents, yields a negative PCR signal for the 1.2kB MOMP gene of C. ***pneumoniae*** . Analysis on agarose gel with ethidium bromide visualization under UV light.

DETD . . . signals using the preferred primers which amplify the full length MOMP gene suggests that mutations in these regions of C. ***pneumoniae*** is rare. Standard conditions for this gene product in a 50-.mu.l volume is 35 cycles with 1 second ramp times. . .

DETD [0239] b. In situ PCR 0.5 This procedure identifies individual cells containing RB and cryptic forms of C. ***pneumoniae*** . Cultured cells are adhered to glass slides with formalin, or formalin fixed tissue sections embedded in paraffin are adhered to. . .

DETD [0243] The full length MOMP gene of C. ***pneumoniae*** was directionally cloned into the pET expression plasmid at the NCOI and NOTI restriction sites using primers to introduce these. . .

DETD . . . length expressed recombinant fusion protein or the modified MOMP following endopeptidase cleavage can be used as the antigen for a ***Chlamydia*** species ELISA. Other expression systems in E. coli or

Baculovirus can be used for synthesis of the MOMP protein as. . .

DETD [0246] The recombinant MOMP-based ELISA described above provides a sensitive method for the quantitation of immunoglobulins against the ***Chlamydia*** genus in serum, plasma, CSF, and other body fluids. In order to provide ELISA assays that are species- and potentially strain-specific for the various ***Chlamydia*** , two regions in the MOMP have been identified which show minimal amino acid sequence homologies and which are predicted by. . . parallels that described above for the recombinant MOMP-based ELISA. In addition, a highly antigenic domain (FIG. 6) common to all ***Chlamydia*** has been identified and was developed as an alternative genus-specific ELISA for the ***Chlamydia*** . Immunization of rabbits has verified the antigenicity of each peptide to each peptide (Table 9). Monoclonal antibodies have further verified. . . analysis of the nucleotide-generated amino acid sequence of each species-specific MOMP.

TABLE 9

Antigenic Responses To Peptides From 4 Species of ***Chlamydia*** Identified By Hydrophilicity And Peptide Movement As Highly Antigenic

Chlamydia Species	Peptide.sup.b	Titer.sup.a Pre	Post
C. ***pneumoniae***	90-105	100	>3200
C. ***trachomatis*** L2	91-106	800	>3200
C. psittaci	92-106	400	>3200
C. ***trachomatis*** (mouse)	89-105	0	>3200
C. ***pneumoniae***	158-171	25	>3200
C. ***trachomatis*** L2	159-175	200	>3200
C. psittaci	160-172	100	>3200
C. ***trachomatis*** (mouse)	158-171	800	>3200
C. ***pneumoniae***	342-354	200	>3200
C. ***trachomatis*** L2	342-354	100	>3200
C. psittaci	ND.sup.c		
C. ***trachomatis*** (mouse)	ND.sup.c		

.sup.aReciprocal titer

.sup.bImmunogenic peptide and ELISA antigen of specific amino acid sequence against the indicated pre-immunization and post-immunization rabbit. .

.

DETD [0247] Table 10 illustrates reciprocal titers of a polyclonal and monoclonal antibody against C. ***trachomatis*** cross-reactive against C. ***pneumoniae*** peptide encompassing amino acids 342-354 and a recombinant full length MOMP from C. ***pneumoniae*** .

TABLE 10

Reciprocal titers of a polyclonal and a monoclonal antibody against C. ***trachomatis*** cross-reactive against C. ***pneumoniae*** peptide encompassing amino acids 342-354 and a recombinant full length MOMP from C. ***pneumonia*** .

Antigen	Titer.sup.a Polyclonal Ab.sup.b	Monoclonal Ab.sup.c

CPN Momp.sup.d	400	0
CPN 90-105.sup.e	50	0
CPN 158-171.sup.f	50	0
CPN 342-354.sup.g	>3200	1600

.sup.aReciprocal titer
.sup.bPolyclonal goat Ab from Chemicon Inc. against MOMP of C. ***trachomatis***
.sup.cMonoclonal Ab (ICN, Inc.) against MOMP of C. ***trachomatis***
.sup.dC. ***pneumoniae*** recombinant MOMP
.sup.eAmino acid peptide 90-105 of C. ***pneumoniae***
.sup.fAmino acid peptide 158-171 of C. ***pneumoniae***
.sup.gAmino acid peptide 342-354 of C. ***pneumoniae***
DETD [0250] C. ***pneumoniae*** EBs were grown in primary human umbilical vein endothelial cells (HuEVEC; early passage), HeLa 199, or a suitable alternative in. . .
DETD [0252] Western blots were prepared by SDS-PAGE of C. ***pneumoniae*** EBs (non-formalin fixed) harvested from infected HuEVEC or HeLa cell lysates, electrophoresed under standard SDS-PAGE conditions, and transferred to nitrocellulose. . .
DETD [0255] In Vitro Antimicrobial Susceptibility Testing for C. ***pneumoniae***
DETD [0256] Tissue culture cells containing cryptic phase C. ***pneumoniae*** (H-292, HeLa, HEL, HuEVEC, McCoy, etc.) are plated at subconfluency in a 96-well microtiter plate (flasks or plates or other. . .
DETD . . . at 1 .mu.g/ml failed to clear HeLa cells in culture of a detectable PCR signal for the MOMP gene of ***Chlamydia*** ***pneumoniae*** . In contrast, triple agents consisting of isoniazid (INH), metronidazole, and penicillamine (1 .mu.g/ml each) resulted in no detectable PCR signal. . .
DETD . . . methodology described in the section above entitled "Methodology for Selecting Potential Agent Combinations".
TABLE 11

Susceptibility to Antibiotics for Cryptic C. ***pneumoniae*** Cultured in HeLa Cells.sup.a

Antibiotic	Conc (.mu.g/ml)	PCR.sup.b
Ofloxacin	1	positive
Clarithromycin	1	positive
INH	1	positive
Metronidazole	1	positive
Penicillamine. . .		

DETD [0259]
TABLE 12

Susceptibility to Antibiotics by PCR for Cryptic ***Chlamydia*** ***pneumoniae*** Cultured in HeLa Cells.sup.1

Phase of the Chlamydial Life Cycle	
EB (Extracellular EB .fwdarw. RB Stationary Phase RB	RB .fwdarw. EB. . .

DETD . . . typical responses to combination antibiotic therapy in a

variety of patients with diagnostic evidence of an active infection by C. ***pneumoniae*** . Unlike typical immune responses to infection with infectious agents, most of the included patients have not only detectable IgM titers. . . the IgM titers generally fall, with a rise in IgG titer (as expected). Current methods of detecting antibodies against C. ***pneumoniae*** (Indirect immunofluoresence, MIF) are incapable of accurately identifying high IgM titers against C. ***pneumoniae*** . Moreover, current procedures are genus specific and not species specific as are peptide-based ELISAs.

DETD . . . months with an EDSS = 8.0 (triplegic plus speech and swallowing impairments). A positive CSF PCR and culture for C. ***pneumoniae*** led to treatment with combination antibiotics. The patient improved on all spheres of neurologic function over the following six months.. . . legs. Over 5 months his EDSS score worsened from 7.0 to 8.0. His CSF was positive by PCR for C. ***pneumoniae*** and he was placed on combination antibiotics. Over the next six months he gradually improved in his balance, coordination and. . . to response to corticosteroids on two successive occasions. Five months later, his EDSS score was 7.5. Following a positive C. ***pneumoniae*** PCR he was placed on combination antibiotics. He has gradually gained strenght in his lower extremities and five months later. . . progressive MS with recent progressive bulbar symptoms, axtaxia, and paraplegia (EDSS = 8.5). PCR for the MOMP gene of C. ***pneumoniae*** in the CSF was positive. She was placed on combination antibiotics with no further progression of symptoms for the last. . . ulcers improved again.

TW PG Severe PG, initiated after a chemical burn in 1991, but with PCR negative serology for C. ***pneumoniae*** . Patient did not initially respond to combination antibiotic therapy. A positive biopsy culture for C. ***pneumoniae*** resulted in the recent re-institution of combination antibiotics. However, after no . improvement, patient went off therapy.

AM IBD Row 5 This. . . the colectomy, the patient experienced neurological symptoms, fatigue, myalgias, arthralgias, and an acneoform skin rash. Serology was performed for C. ***pneumoniae*** and was positive with an IgM of 1:3200, IgG 1:400 and PCR positive. Therapy with combination antibiotics was initiated. After. . . resolution

of her proclitis on visual exam.
NM CFS Vanderbilt University initial patient that resulted in our first association of C.
pneumoniae , initially complained of the insidious onset of debilitating fatigue.
This was associated with a severe cognitive dysfunction that disrupted his. . . Infectious Disease Clinic at
Vanderbilt no definitive or presumptive diagnosis could be made. A subsequent
high antibody titer against C. ***pneumoniae*** led to standard anti-chlamydial
antibiotic therapy over a three month period with gradual disappearance of
fatigue and cognition symptoms. On. . . developed acute anxiety attacks relieved by anti-
porphyrin therapy.
WM CF Row 7 CF following acute stress. Pre-illness serum negative for anti- ***Chlamydia***
pneumoniae antibodies which peaked six weeks following stress. Pre-illness PCR
was weak positive that became strongly positive. On combination antibiotic
therapy. . .
DETD [0269] A set of mice were tested for infection with C. ***pneumoniae*** . Of 10 mice tested, 8 (80%) were PCR positive for C. ***pneumoniae*** . The mice were then placed on triple-antibiotic therapy: Amoxicillin, Metronidazole and INH at 50 .mu.g/ml each in their water.
DETD [0273] Patents with systemic infections caused by C. ***pneumoniae*** were evaluated for secondary porphyria. The presence of enzymes (i.e., .DELTA.-ALA synthase and PBG deaminase) for heme biosynthesis were determined. . . The results are reported in Table 14.
TABLE 14

Examples of Secondary Porphyria in Patients with Systemic infections caused by C. ***pneumoniae*** .sup.a
Enzymes of Heme biosynthesis.sup.b
Patient ALA PBG Elevated Fecal Porphyrins (24 hr) Elevated Urinary Porphyrins (24 hr)
ID synthase deaminase Porphyrin. . .
DETD [0275] Patients with systemic infections caused by C. ***pneumoniae*** were tested for the presence of antibodies to porphyrin ring structures (i.e., vitamin B12, coproporphyrinogen--III, protoporphyrin, porphyrobelinigen and .sup.a-ALA). IgM. . .
DETD . . . below in Table 15.
TABLE 15

Examples of Antibody Titers.sup.a to Porphyrin Ring Structures in Patients with Systemic infections caused by C. ***pneumoniae***

Patient	B12		Copro III		Protoporphyrin		Porphyrobelinigen -ALA	
ID	IgM	IgG	IgM	IgG	IgM	IgG	IgM	IgG

IgM IgG

KRH 1:640 1:160 1:640 1:160. . .
DETD SEQUENCE CHARACTERISTICS:
LENGTH: 14 amino acids
TYPE: amino acid
STRANDEDNESS: single
TOPOLOGY: linear
MOLECULE TYPE: peptide
SEQUENCE: 100
Cys ***Ile*** ***Gly*** ***Leu*** ***Ala***
Gly ***Thr*** ***Asp*** ***Phe*** ***Ala***
Asn ***Gln*** ***Arg*** ***Pro***
1 5 10
CLM What is claimed:
. . . each of which is effective against a different phase of chlamydial life cycle, until the biological material is negative for ***Chlamydia*** according to a test that detects elementary body phase ***Chlamydia*** , replicating phase ***Chlamydia*** , and cryptic phase ***Chlamydia*** , thereby treating said Alzheimer's disease.

11. The method of claim 1, wherein the test that detects elementary body phase ***Chlamydia*** , replicating phase ***Chlamydia*** , and cryptic phase ***Chlamydia*** comprises a step of nucleic acid amplification.

. . . said mammal an antichlamydial agent, wherein said antichlamydial agent inhibits infection of cells or inhibits growth or replication of C. ***pneumoniae*** in said mammal, thereby treating said Alzheimer's disease.

. . . and an anti- inflammatory agent, wherein said antchlamydial agent inhibits infection of cells or inhibits growth or replication of C. ***pneumoniae*** in said human, thereby treating said Alzheimer's disease.

L7 ANSWER 4 OF 4 USPATFULL on STN
AN 2003:161945 USPATFULL
TI Diagnosis and management of infection caused by ***chlamydia***
IN Mitchell, William M., Nashville, TN, United States
Stratton, Charles W., Nashville, TN, United States
PA Vanderbilt University, Nashville, TN, United States (U.S. corporation)
PI US 6579854 B1 20030617
AI US 1998-73661 19980506 (9)
RLI Continuation-in-part of Ser. No. US 1998-25174, filed on 18 Feb 1998 Continuation-in-part of Ser. No. US 1998-25521, filed on 18 Feb 1998, now abandoned Continuation-in-part of Ser. No. US 1998-25176, filed on 18 Feb 1998, now patented, Pat. No. US 6258532 Continuation-in-part of Ser. No. US 1997-911593, filed on 14 Aug 1997, now abandoned
PRAI US 1997-45689P 19970506 (60)
US 1997-45739P 19970506 (60)
US 1997-45779P 19970506 (60)
US 1997-45780P 19970506 (60)
US 1997-45787P 19970506 (60)

US 1996-23921P 19960814 (60)
DT Utility
FS GRANTED
EXNAM Primary Examiner: Weddington, Kevin E.
LREP Clark & Elbing LLP
CLMN Number of Claims: 9
ECL Exemplary Claim: 1
DRWN 6 Drawing Figure(s); 4 Drawing Page(s)
LN.CNT 4353
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The present invention provides a unique approach for the diagnosis and management of infections by ***Chlamydia*** species, particularly C. ***pneumoniae*** . The invention is based, in part, upon the discovery that a combination of agents directed toward the various stages of the chlamydial life cycle is effective in substantially reducing infection. Products comprising combination of antichlamydial agents, novel compositions and pharmaceutical packs are also described.
TI Diagnosis and management of infection caused by ***chlamydia***
AB The present invention provides a unique approach for the diagnosis and management of infections by ***Chlamydia*** species, particularly C. ***pneumoniae*** . The invention is based, in part, upon the discovery that a combination of agents directed toward the various stages of. .
.
SUMM . . . members of the genus Chlamydiae induces a significant inflammatory response at the cellular level. For example, genital lesions produced by ***Chlamydia*** ***trachomatis*** frequently elicit a vigorous influx of lymphocytes, macrophages, and plasma cells, suggesting the development of humoral and cellular immunity. Yet, clinically, the initial infection is frequently varied in symptomatology and may even be asymptomatic. Once fully established, the ***Chlamydia*** are difficult to eradicate, with frequent relapse following antibiotic therapy. Evidence also indicates that the ***Chlamydia*** may become dormant and are then shed in quantities too few to reliably detect by culture.
SUMM ***Chlamydia*** ***pneumoniae*** (hereinafter "C. ***pneumoniae*** ")) is the most recent addition to the genus Chlamydiae and is isolated from humans and currently is recognized as causing approximately 10 percent of community acquired cases of ***pneumonia*** (Grayston et al., J. Inf. Dis. 161:618-625 (1990)). This newly recognized pathogen commonly infects the upper and lower respiratory tract and is now recognized as ubiquitous in humans. C. ***pneumoniae*** is well-accepted as a human pathogen that may be difficult to eradicate by standard antibiotic therapy (Hammerschlag et al., Clin. Infect. Dis. 14:178-182 (1992)). C. ***pneumoniae*** is known to persist as a silent or mildly symptomatic pathogen, resulting in a chronic, persistent infection (J. Schacter, In: Baun AL, eg. Microbiology of ***Chlamydia*** , Boca Raton, Fla., CRC Press, 1988, pp. 153-165).
SUMM The current therapy for suspected/confirmed C. ***pneumoniae*** infection is with a short course (e.g., 2-3 weeks) of a single antibiotic. C. ***pneumoniae*** is susceptible in vitro to tetracyline, erythromycin, clarithromycin, and fluoroquinolones such as ofloxacin and sparfloxacin (Kuo et al., Antimicrob Agents. . . Agents Chemother 38:1873-1878 (1994); M. R. Hammerschlag, Infect. Med. pp. 64-71 (1994)). Despite this demonstration of in vitro susceptibility, C.

pneumoniae infections may relapse following antibiotic therapy with these agents. In vitro studies on the persistence of Chlamydiae despite specific and. . .

SUMM . . . diagnosis of pathogenic infection as well as therapeutic approaches to manage the infection. Due to the highly infective nature of ***Chlamydia*** EBs and their ability to reinfect cells, there is also a need for antichlamydial therapy which totally eradicates this pathogen,. . .

SUMM The present invention provides a unique approach for the diagnosis and management of infection by ***Chlamydia*** species, particularly C. ***pneumoniae*** . The invention is based upon the discovery that a combination of agents directed toward many of the various stages of. . . ultimately prevent reinfection/reactivation of the pathogen. Accordingly, one embodiment of the invention pertains to methods of treating infection by a ***Chlamydia*** species, comprising administering to an individual in need thereof a combination of antichlamydial agents, comprising at least two agents, each. . .

SUMM . . . Use of the combination of antichlamydial agents or compositions thereof for the manufacture of a medicament for the management of ***Chlamydia*** infection is also described. In a particular embodiment, the agents can be assembled individually, admixed or instructionally assembled.

SUMM . . . during a course of therapy, the invention provides a means for packaging therapeutic agents, described herein, for the management of ***Chlamydia*** infection. For example, a pack can comprise at least two different agents, each of which is targeted against a different. . .

SUMM . . . the infection status of an individual and/or the progress of therapy in an individual undergoing therapy for infection caused by ***Chlamydia*** . The method comprises quantifying antibody titer or other measure to the pathogen and comparing the measure to antibody measure quantified. . . of the therapy. The invention also pertains to a method for monitoring the course of therapy for treating infection by ***Chlamydia*** , comprising determining presence or absence of ***Chlamydia*** in an infected individual at time intervals during course of therapy. In a particular embodiment, this is determined by PCR. . .

SUMM Detection of the presence of ***Chlamydia*** in a sample of biological material taken from an individual thought to be infected therewith is important in determining the. . . therapy and the agents to be used. This can be achieved by detecting the presence of DNA encoding MOMP of ***Chlamydia*** or other chlamydial genes in the individual. In one aspect of the invention, diseases associated with ***Chlamydia*** infection, such as inflammatory diseases, autoimmune diseases and diseases in which the individual is immunocompromised, can be treated by managing (i.e., significantly reducing infection or eradicating) the ***Chlamydia*** infection using the novel approach described herein. Both clinical and serological improvements/resolutions in patient status have been demonstrated.

SUMM . . . agent(s) capable of significantly reducing/eliminating chlamydial infection. The method comprises preparing tissue culture from cell lines; inoculating these cells with ***Chlamydia*** in the absence of cycloheximide; allowing the ***Chlamydia*** to infect these cells for several days; adding agent(s) to be tested, which agent(s) is/are replaced as needed for the. . . suitable nucleotide

amplification assay, such as PCR. Preferably the presence or absence of signal for amplified DNA encoding MOMP of ***Chlamydia*** or other chlamydial protein is determined. Absence of a signal indicates a reduction in the degree of infection below that. . . are particularly useful as a drug screening tool for assessing the activity of single agents or combinations of agents against ***Chlamydia*** infection.

SUMM In one embodiment, a suitable nucleotide assay for identifying agents effective against a cryptic form of ***chlamydia*** comprises, in the presence of agent(s) to be tested, is performed by subjecting cultured cells to protease/reducing agent (e.g., dithiotreitol. . . treated solution; exposing DNA to appropriate polymerase, dNTPs and primers for DNA amplification of MOMP or other protein of the ***Chlamydia*** species; and determining the presence or absence of amplified DNA by visualizing the ethidium bromide treated DNA product by gel electrophoresis, for example. In particular embodiments, the ***Chlamydia*** species is C. ***pneumoniae*** and the appropriate primers are CHLMOMPDB2 and CHLMOMPCB2.

SUMM The invention further relates to a method of identifying cells containing a cryptic form of a ***Chlamydia*** species by a nucleic acid amplification technique (e.g., PCR) comprising subjecting cultured cells to protease digestion; stopping protease activity; exposing. . . heat-stable DNA polymerase, dNTPs and labeled primers (e.g., 3'-biotin labeled, 5'-biotin labeled) for amplification of DNA encoding MOMP of the ***Chlamydia*** species; washing the cells; exposing the cells to a reporter molecule (e.g., strepavidin-conjugated signal enzyme); exposing the cells to an. . .

SUMM A method of identifying cells containing a cryptic form of ***Chlamydia*** comprises treating cultured cells, thought to be infected with ***Chlamydia*** , with a disulfide reducing agent; subjecting cultured cells to protease digestion; exposing cells to appropriate polymerase, dNTPs and primers for. . . exposing the cells to an appropriate substrate for the reporter enzyme; and determining the presence of a cryptic form of ***Chlamydia*** by visualizing the amplified DNA encoding a chlamydial protein. Preferably the amplification technique is PCR and the primers are CHLMOMPDB2 and CHLMOMPCB2 of ***Chlamydia*** ***pneumoniae*** .

SUMM . . . of Chlaniydia. Accordingly, the method comprises treating cultured cells grown in the absence of cycloheximide, thought to be infected with ***Chlamydia*** , with a disulfide reducing agent; allowing the ***chlamydia*** to replicate; adding a test agent; subjecting cultured cells to protease digestion; exposing cells to appropriate polymerase, dNTPs and primers. . . exposing the cells to an appropriate substrate for the reporter enzyme; and determining the presence of a cryptic form of ***Chlamydia*** by visualizing the amplified DNA encoding a chlamydial protein, such as MOMP.

SUMM The present invention pertains to methods for clearing biological material infected with ***Chlamydia*** to produce ***Chlamydia*** -free cell lines and animals, and to methods of maintaining biological material, e.g, cell lines and animals, such that they remain ***Chlamydia*** -free. According to the method, a biological material is cleared from ***Chlamydia*** infection by contacting the biological material with at least two agents but preferably three agents, each of which is targeted against a different phase of the chlamydial life cycle, until the biological material no longer tests positive for ***Chlamydia*** . The agents can be selected from the

group consisting of a) agents targeted against a cryptic phase of the chamydial. . .

SUMM Biological material that has been cleared of ***Chlamydia*** infection, according to the methods of this invention, are also described. The biological material can be a continuous cell line such as HeLa-CF, HL-CF, H-292-CF, HuEVEC-CF and McCoy-CF; wherein "CF" is a shorthand annotation for " ***Chlamydia*** -free"). Alternatively, the biological material can be an animal, such as a mouse, rabbit or other animal model, which is negative for ***Chlamydia*** .

SUMM The invention also pertains to methods of maintaining a ***Chlamydia*** -free status in animals and cell lines which have been cleared of ***Chlamydia*** infection by the methods of this invention, or have never been infected, such as their ***Chlamydia*** -free offspring or progeny. Cells or animals can be maintained as ***Chlamydia*** -free by maintaining them on antibiotics and/or treating their nutrients and environment to ensure that they are ***Chlamydia*** -free. Particularly, a source of nutrients to be administered to ***Chlamydia*** -free cells or animals can be treated to inactivate or remove any chlamydial elementary bodies therefrom. This can be accomplished.by exposing. . .

SUMM . . . pertains to a diagnostic kit or pack comprising an assembly of materials selected from the group consisting of antibiotics, reagents, ***Chlamydia*** -free cell lines, and combinations thereof, or other materials that would be necessary to perform any of the methods described herein.

SUMM The invention further relates to a method of detecting viable ***Chlamydia*** in a biological material suspected of being contaminated therewith, comprising culturing ***Chlamydia*** -free cells or animals in the presence of biological material and then determining the presence or absence of viable ***Chlamydia*** in the culture.

SUMM The invention also pertains to a method for differentiating porphyria caused by ***Chlamydia*** species from porphyria caused by a genetic disorder. The method comprises measuring peripheral red blood cell enzymes and/or performing a. . . more components of the heme pathway, the porphyria is not caused by a genetic disorder and may be caused by ***Chlamydia*** . The invention relates to a method for diagnosing secondary porphyria caused by ***Chlamydia*** in an individual having symptoms associated therewith, comprising determining the presence or amount of obligatory enzymes in heme biosynthesis in red blood cells of the individual and determining the presence of ***Chlamydia*** in the individual. The invention further relates to a method for differentiating secondary porphyria caused by ***Chlamydia*** from that caused by a genetic disorder in an individual, comprising treating infection by ***Chlamydia*** at many stages of its life cycle and then assessing whether porphyrins have been reduced, wherein a decrease in the porphyrin levels is indicative that the porphyria is secondary and caused by ***Chlamydia*** .

SUMM . . . can be automated using a computerized system, for example, to formulate a drug therapy for management of infection caused by ***Chlamydia*** . The method comprises determining targets within the chlamydial life cycle, for each determined target, identifying agents that are active against. . . combining at least a subset of the identified agents to provide a combination therapy for management of infection caused by ***Chlamydia*** , the agents in said subset

individually being active against different targets in the life cycle of ***Chlamydia*** . The targets include identifying phases of the chlamydial life cycle and for each identified life cycle phase, determining at least. . .

DRWD FIGS. 1A and 1B show a sequence alignment of various ***Chlamydia*** MOMPs.

DRWD FIG. 3 illustrates the constant and variable domain (VD) of various ***Chlamydia*** species.

DETD . . . or in combination to eliminate or interfere with more than one of the distinct phases of the life cycle of ***Chlamydia*** species. These chlamydial phases include the intracellular metabolizing/replicating phase; the intracellular "cryptic" phases; and the extracellular EB phase. Current concepts. . .

DETD . . . methods for the management of Chiamydia infections are described in detail below. For the purposes of this invention, "management of ***Chlamydia*** infection" is defined as a substantial reduction in the presence of all phases/forms of ***Chlamydia*** in the infected host by treating the host in such a way as to minimize the sequellae of the infection. ***Chlamydia*** infections can thus be managed by a unique approach referred to herein as "combination therapy" which is defined for the. . . or manage chlamydial infection. The diagnostic methods and combination therapies described below are generally applicable for infection caused by any ***Chlamydia*** species, including but not limited to C. ***pneumoniae*** , C. ***trachomatis*** , C. psittaci and C. pecorum. Infections in which the causative agent is C. ***pneumoniae*** are emphasized.

DETD Antichlamydial agents, which have been identified as effective against ***Chlamydia*** by the susceptibility testing methods described herein, can be used singly to affect ***Chlamydia*** in a single stage of its life cycle or as part of a combination therapy to manage ***Chlamydia*** infection. For example, compounds identified as anti-cryptic phase drugs, anti-EB phase drugs, anti-DNA-dependent RNA polymerase drugs and nicotinic acid cogener. . .

DETD Diagnosis of ***Chlamydia*** Infection

DETD The invention pertains to methods for diagnosing the presence of ***Chlamydia*** in a biological material, as well as to the use of these methods to evaluate the serological status of an. . .

DETD . . . more immunoglobulins, such as IgG, IgM, IgA and IgE, against antigenic determinants within the full length recombinant MOMPs of various ***Chlamydia*** species. Detection of IgG and/or IgM against antigenic determinants within the full length recombinant MOMP of C. ***pneumoniae*** is preferred. IgA determinations are useful in the analysis of humoral responses to ***Chlamydia*** in secretions from mucosal surfaces (e.g., lung, GI tract, gerontourinary tract. etc.). Similarly, IgE determinations are useful in the analysis of allergic manifestatins of disease. Table 1 below provides the GenBank Accession numbers of various MOMPs for ***Chlamydia*** species.

DETD

TABLE 1

Species	Strain ID		GenBank Accession No.
C. ***trachomatis***	A	CTL/A	M33636

C. ***trachomatis*** A CTL/A M58938
M33535
C. ***trachomatis*** A CTL/A J03813
C. ***trachomatis*** B CTL/B M33636
C. ***trachomatis*** C CTL/L M17343
M19128
C. ***trachomatis*** D CTL/D A27838
C. ***trachomatis*** E CTL/E X52557
C. ***trachomatis*** F CTL/F X52080
M30501
C. ***trachomatis*** H CTL/H X16007
C. ***trachomatis*** L1 CTL/L1 M36533
C. ***trachomatis*** L2 CTL/L2 M14738
M19126
C. ***trachomatis*** L3 CTL/L3 X55700
C. ***trachomatis*** Mouse Pneumo CTL/MP X60678
C. pecorum Ovine CPC/OP Z18756
Polyarthritis
C. psittaci Strain 6BC CPS/6B X56980
C. psittaci Feline CPS/F X61096
C. ***trachomatis*** Da CTL/DA X62921
S45921
C. ***pneumoniae*** TWAR CPN/HU1 M64064
M34922
M64063
C. ***pneumoniae*** Horse CPN/EQ2 L04982
(? C. pecorum)
C. ***pneumoniae*** TWAR CPN/MS not assigned
C. psittaci Horse CPS/EQ1 L04982
DETD . . . with no cross reactivity to other immunoglobulins (Pharmagen; Clone G20-127, Catalog No. 34152D). Peptide-based immunoassays can be developed which are ***Chlamydia*** specific or provide species specificity, but not necessarily strain specificity within a species, using monoclonal or polyclonal antibodies that are. . .
DETD Recombinant-based immunological assays have been developed to quantitate the presence of immunoglobulins against the ***Chlamydia*** species. Full length recombinant ***Chlamydia*** MOMP can be synthesized using an appropriate expression system, such as in E. coli or Baculovirus. The expressed protein thus. . . for suitable immunological methods, as discussed above. Protein-based immunological techniques can be designed that are species- and strain-specific for various ***Chlamydia*** .
DETD Diagnosis of chlamydial infection can now be made with an improved IgM/IgG C. ***pneumoniae*** method of quantitation using ELISA techniques, Western blot confirmation of ELISA specificity and the detection of the MOMP gene of C. ***pneumoniae*** in serum using specific amplification primers that allow isolation of the entire gene for analysis of expected strain-specific differences.
DETD . . . (PCR) methodologies which comprise solution PCR and in situ PCR, to detect the presence or absence of unique genes of ***Chlamydia*** . Species-specific assays for detecting Chlarnydia can be designed based upon the primers selected. Examples of suitable PCR amplification primers are. . .
DETD . . . CTL/L1 ATGAAAAAACTCTTGAAATCGGTATTAGTGTTTGCCGCTTTGAGTTCTGC 16
M14738/M19126 CTL/L2 ATGAAAAAACTCTTGAAATCGGTATTAGTGTTTGCCGCTTTGAGTTCTGC 17

X55700 CTL/L3 ATGAAAAAACTCTTGAAATCGGTATTAGTGTTTGCCGCTTTGAGTTCTGC 18
X60678 CTL/MP ATGAAAAAACTCTTGAAATCGGTATTAGCATTTGCCGTTTTGGGTTCTGC 19

Chlamydial SEQ ID
Species Strain ID Terminal Fifty Nucleotides NO.

C. ***pneumoniae*** TWAR CPNHU1
GTTTAATTAACGAGAGAGCTGCTCACGTATCTGGTCAGTTCAG
ATTCTAA 20
C. ***pneumoniae*** MS CPNHU2
GTTTAATTAACGAGAGAGCTGCTCACGTATCTGGTCAGTTCAGAT
TCTAA 21
C. psittaci Horse CPNEQ1 CAACGTTAATCGACGCTGACAAATGGTCAATCACTGGTGAAGCACGCTTA 22
C. ***pneumoniae*** Horse CPNEQ2 GTTTAATTAACGAGAGAGCTGCTCACATATCTGGTCAGTTCA
GATTCTAA 23
C. psittaci SBE CPS/6B AACGTTAATCGACGCTGACAAATGGTCAATCACTGGTGAAGCACGCTTAA 24
C. psittaci Ewe CPS/AB1 AACGTTAATCGACGCTGACAAATGGTCAATCACTGGTGAAGCACGCTTAA 25
abortion
C. psittaci Bovine CPS/AB2 GCTTAATCAATGAAAGAGCCGCTCACATGAATGCTCAATTCAGATTCTAA
26
abortion
C. psittaci Avian CPS/AV/C GCTTAATCAATGAAAGAGCTGCTCACATGAATGCTCAATTCAGATTCTAA
27
C. psittaci Feline CPS/F GCTTAATCGACGAAAGAGCTGCTCACATTAATGCTCAATTCAGATTCTAA 28
C. ***trachomatis*** Hu/A CTL/A CGCAGTTACAGTTGAGACTCGCTTGATCGATGAGAGAGCAGCT
CACGTAA 29
C. ***trachomatis*** Hu/C CTL/C GCTTGATCGATGAGAGAGCAGGTCACGTAAATGCACAATTCCG
GTTCTAA 30
C. ***trachomatis*** Hu/Da CTL/DA GCTTGATCGATGAGAGAGCAGCTCACGTAAATGCACAATTC
CGCTTCTAA 31
C. ***trachomatis*** HU/E CTL/E CGCTTGATCGATGAGAGACTGCTCACGTAAATGCACAATTCCG
CTTCTAA 32
C. ***trachomatis*** Hu/F CTL/F GCTTGATCGATGAGAGAGCTGCTCACGTAAATGCACAATTCCG
CTTCTAA 33
C. ***trachomatis*** Hu/H CTL/H GCTTGATCGATGAGAGAGCAGCTCACGTAAATGCACAATTCCG
CTTCTAA 34
C. ***trachomatis*** Hu/L1 CTL/Li GCTTGATCGATGAGAGAGCTGCTCACGTAAATGCACAATTC
CGCTTCTAA 35
C. ***trachomatis*** Hu/L2 CTL/L2 GCTTGATCGATGAGAGAGCTGCTCACGTAAATGCACAATTC
CGCTTCTAA 36
C. ***trachomatis*** Hu/L3 CTL/L3 GCTTGATCGATGAGAGAGCAGCTCACGTAAATGCACAATTC
CGCTTCTAA 37
C. ***trachomatis*** Mouse CTL/MP GCTTGATCGATGAAAGAGCAGCTCACGTAAATGCTCAGTTC
CGTTTCTAA 38

.sup.aSequence from a cerebral spinal fluid of a patient with multiple
sclerosis isolated by the inventors. Sequence is identical to TWAR C.
pneumoniae with exception of a C/T mutation at NT 54 and a G/A
mutation at NT 126.
.sup.bTerminator condon underlined
DETD
TABLE 3

Primers for PCR Amplification of Entire MOMP Gene.sup.a
Chlamydia SEQ ID

Species Strain ID Sequence T.sub.m.sup.b NO.

Plus Strand Primer
C. ***pneumoniae*** TWAR CHLMOMP ATGAAAAAAC TCTTAAAGTC GGCGTTATTA
61.4.degree. 105
DB2 TCCGCCGC
C. ***trachomatis*** L2 CTMOMP ATGAAAAAAC TCTTGAAATC GGTATTAGTG
61.2.degree. 106
L2DB TTTGCCGCTT TGAG
C. psittaci Feline PSOMP ATGAAAAAAC TCTTAAAATC GGCATTATTA 62.1.degree. 107
FPN-D TTTGCCGCTG CGGG
C. psittaci 6BC PSOMP ATGAAAAAAC TCTTGAAATC GGCATTATTG 63.0.degree. 108
6BC-b TTTGCCGCTA CGGG
C. ***trachomatis*** Mouse CTMU ATGAAAAAAC TCTTGAAATC GGTATTAGCA
63.5.degree. 109
MOMP-D TTTGCCGTTT TGGGTTCTGC
Minus Strand Primer
C. ***pneumoniae*** TWAR CHLMOMP TTAGAATCTG AACTGACCAG ATACGTGAGC
64.4.degree. 110
CB2 AGCTCTCTCG
C. ***trachomatis*** L2 CTMOMP TTAGAAGCGG AATTGTGCAT TTACGTGAGC
61.5.degree. 111
L2CB AGCTC
C. psittaci Feline PSOMP TTAGAATCTG AATTGAGCAT TAATGTGAGC 62.2.degree. 112
FPN_C AGCTCTTTCG TCG
C. psittaci 6BC PSOMP TTAGAATCTG AATTGACCAT TCATGTGAGC 63.4.degree. 113
GBC_C AGCTCTTTCA TTGATTAAGC G
C. ***trachomatis*** Mouse CTMU TTAGAAACGG AACTGAGCAT TTACGTGAGC
63.2.degree. 114
MOMP_C TGCTCTTTCA TC

.sup.aAll primers amplify under identical amplification conditions: 94.degree.
C. for 1. . .
DETD . . . clinical management of the chlamydial infection. Serological
improvement can be based upon the current serological criteria for
eradication of chronic ***Chlamydia*** reported below in Table 4.
DETD
TABLE 4

Serological Criteria for Eradication
of Chronic ***Chlamydia*** ***pneumoniae*** Infection

IgM .ltoreq.1:25
IgG Stable titer 1:100
PCR Negative
DETD . . . bromide staining and UV light detection. PCR primers can be
designed to selectively amplify DNA encoding MOMP of a particular
Chlamydia species, such as the MOMP of C. ***pneumoniae*** ,
C. pecorum, C. ***trachomatis*** , C. psittaci (See FIG. 1). Primers
that are from about 15-mer to about 40-mer can be designed for this
purpose.
DETD Clearing and Maintaining ***Chlamydia*** -free Organisms
DETD The present invention provides a unique approach for creating and
maintaining animals and cell lines which are free of ***Chlamydia***

infection. Also described herein are methods for creating nutrients and culture media that are suitable for use with animals and cell lines that have been cleared of ***Chlamydia*** infection.

DETD Attempts to culture isolates of C. ***pneumoniae*** from blood and cerebrospinal fluid (CSF) have resulted in the discovery that the continuous cell lines routinely used to cultivate C. ***pneumoniae*** are cryptically infected with C. ***pneumoniae*** . These include not only in house stocks of HeLa, HL, H-292, HuEVEC and McCoy cells, but also stocks obtained from. . . for HL cells, as well as a commercial supplier (Bartells) of H-292 and McCoy cells for the clinical culture of ***Chlamydia*** . The presence of a cryptic form of C. ***pneumoniae*** in these cells has been repeatedly demonstrated by solution PCR amplifying the MOMP. In situ PCR in HeLa cells against. . . be present in 100% of cells. Nevertheless, fluoroscenated mAb to LPS in McCoy cells does not yield any indication of ***Chlamydia*** (i.e., reactive against all Chiamydia) while fluoroscenated mAb to C. ***pneumoniae*** MOMP yields a generalized fluorescence throughout the cytoplasm that can be confused with non-specific autofluorescence. Infection with ***Chlamydia*** ***trachomatis*** (Bartells supply) yields the typical inclusion body staining with the LPS mab (i.e., cross reactive with all species of ***Chlamydia***) with no change in cytoplasmic signal with anti-MOMP mAb against C. ***pneumoniae*** . These findings (solution PCR, in situ PCR, mAb reactivity) were interpreted as consistent with a cryptic (non-replicating) infection by C. ***pneumoniae*** of cells commonly used to culture the organism. Further, virtually all untreated rabbits and mice tested to date have PCR signals for the C. ***pneumoniae*** MOMP gene.

DETD This creates a currently unrecognized problem of major significance for those clinical labs providing C. ***pneumoniae*** culture services as well as investigators who now do not know whether their results in animals or in cell culture. . . by cryptic chlamydial contamination. Clinical and research laboratories currently have no way to determine whether an organism is, in fact, ***Chlamydia*** -free.

DETD This invention pertains to a method for clearing cells and animals of C. ***pneumoniae*** and keeping them clear. Clearing them entails contacting the infected organism with agents used singly or in combination to eliminate or interfere with more than one of the distinct phases of the life cycle of ***Chlamydia*** species. Keeping them clear entails either maintaining them on antibiotics and/or treating their nutrients and environment to ensure they are ***Chlamydia*** -free. In a preferred embodiment, maintenance conditions comprise a combination of isoniazid (INH) (1 .mu.g/ml), metronidazole (1 .mu.g/ml), and dithiothreitol (10. . .

DETD These techniques have now made it possible to create a variety of ***Chlamydia*** -free (CF) organisms, including continuous cell lines called HeLa-CF, HL-CF, H-292-CF, HuEVEC-CF, McCoy-CF, African green monkey and other cell lines that. . .

DETD Because ***Chlamydia*** is highly infectious, organisms which have been cleared of extracellular, replicating and cryptic infections must be protected from exposure to. . . have discovered that many of the nutrients and other materials used to maintain continuous cell lines are contaminated with viable ***Chlamydia*** EBs. For example, every lot of fetal calf serum has tested positive for the ***Chlamydia*** MOMP gene by PCR. Since extensive digestion is required for isolation of the

DNA, we have concluded it is bound in EBs. C. ***pneumoniae*** can also be cultured directly from fetal calf serum. Thus, it is necessary to inactivate EBs in these materials, such as culture media and nutrients, used to maintain the ***Chlamydia*** -free status of the organism. Collectively these materials are referred to herein as "maintenance materials"). In one embodiment, nutrients and culture media are subjected to gamma irradiation to inactivate ***Chlamydia*** therein. Preferably, the material should be irradiated for a period of time sufficient to expose the material to at least. . . reducing agent, preferably dithiothreitol (e.g., about 10 .mu.M concentration), before the materials are passed through a filtration system to remove ***Chlamydia*** therefrom.

DETD In order to insure that research tools, such as cell lines and animals, remain ***Chlamydia*** -free, an assay has been designed to evaluate whether an organism is ***Chlamydia*** -free. The method comprises obtaining a sample of cells or tissue culture; optionally culturing the cells in the presence or absence of cycloheximide; and determining the presence or absence of ***Chlamydia*** nucleic acid by a suitable amplification technique, such as PCR. The absence of nucleic acid amplification signal is indicative that. . .

DETD Susceptability Testing for Evaluating Active Agents Against Various Forms of ***Chlamydia***

DETD This invention pertains to novel approaches for the susceptibility testing of ***Chlamydia*** species that are necessitated by the complex life cycle of the chlamydial pathogen as well as by its diverse, extensive,. . .

DETD . . . to successfully and totally eradicate chronic chlamydial infections. This is because the current susceptibility testing methods measure only replication of ***chlamydia*** and ignores the well-known "cryptic phase" in which intracellular Chlamydiae are not actively replicating. Moreover, it has also been discovered. . .

DETD . . . the invention pertains to methods for evaluating the susceptibility of the distinct phases and stages of the life cycle of ***Chlamydia*** , particularly the cryptic phase to a particular agent(s), since prior techniques have failed, heretofore, to appreciate the need for drugs that can clear infected cells of cryptic ***Chlamydia*** . A preferred drug screening method which accomplished this objective utilizes tissue culture cells which are maintained, in the absence of. . . in order to encourage cryptic infection. Cryptic infection is uncommon in cells used in standard cell culture susceptibility techniques because ***Chlamydia*** in cycloheximide-paralyzed cells need not compete with the host cell for metabolites and hence are encouraged to replicate.

DETD . . . or combination of compounds to be evaluated as an antichlamydial agent for its ability to significantly reduce the presence of ***Chlamydia*** in living cells. For example, a test agent can include, but is not limited to, antibiotics, antimicrobial agents, antiparasitic agents,. . . as PCR) are used to ascertain the presence or absence of signal for chlamydial DNA encoding MOMP or another unique ***Chlamydia*** gene to determine whether the test agent or combination of agents is/are effective in reducing ***Chlamydia*** infection. The loss of signal (i.e., below the detectable level of the nucleic acid amplification technique) in cells with antibiotic(s) versus its presence in controls is an indication of efficacy of the agent or combination of agents against ***Chlamydia***

.

DETD . . . of this invention can be used to identify an agent or agents which are targeted against any particular species of ***Chlamydia*** and can be used to identify agent(s) targeted against the cryptic form of the pathogen, i.e., is capable of inhibiting. . . embodiment, this is done by performing the susceptibility test while placing the cells under stringent environmental conditions known to induce ***Chlamydia*** to enter a cryptic phase. Agents that are effective against ***Chlamydia*** , as ascertained by the susceptibility testing protocols described herein, can be used as part of a therapy for the management of ***Chlamydia*** infections. Suitable therapeutic protocols are described in detail below, with a particular focus on targeting agents toward specific stages of. . .

DETD In one embodiment, a suitable nucleic acid assay for identifying agents effective against the cryptic form of ***Chlamydia*** comprises, in the presence of agent(s) to be tested, subjecting cultured cells to reducing agent (e.g., dithiotreitol) and protease digestion. . . treated solution; exposing DNA to appropriate polymerase, dNTPs and primers for DNA amplification of MOMP or other protein of the ***Chlamydia*** species; and determining the presence or absence of amplified DNA by visualizing the ethidium bromide treated DNA product by gel electrophoresis, for example, or alternatively by Southern Blot. In particular embodiments, the ***Chlamydia*** species is C. ***pneumoniae*** and the appropriate primers are CHLMOMPDB2 and CHLMOMPCB2.

DETD The invention further relates to a method of identifying cells containing a non-EB cryptic form of a ***Chlamydia*** species by a nucleic acid amplification technique (e.g., PCR) comprising subjecting cultured cells to protease digestion; stopping protease activity; exposing. . . heat-stable DNA polymerase, dNTPs and labeled primers (e.g., 3'-biotin labeled, 5'-biotin labeled) for amplification of DNA encoding MOMP of the ***Chlamydia*** species; washing the cells; exposing the cells to a reporter molecule (e.g., strepavidin-conjugated signal enzyme); exposing the cells to an. . .

DETD The invention pertains to a method of identifying cells containing a cryptic form of ***Chlamydia*** . The method comprises treating cultured cells, thought to be infected with ***Chlamydia*** , with a disulfide reducing agent; subjecting cultured cells to protease digestion; exposing cells to appropriate polymerase, dNTPs and primers for. . . exposing the cells to an appropriate substrate for the reporter enzyme; and determining the presence of a cryptic form of ***Chlamydia*** by visualizing the amplified DNA encoding a chlamydial protein. Preferably, the amplification technique is PCR and the primers are CHLMOMPDB2 and CHLMOMPCB2 of ***Chlamydia*** ***pneumoniae***

.

DETD . . . similar method can be used as an assay for identifying an agent which is effective against a cryptic form of ***Chlamydia*** . Accordingly, the method comprises treating cultured cells grown in the absence of cycloheximide, thought to be infected with ***Chlamydia*** , with a disulfide reducing agent; allowing the ***Chlamydia*** to replicate; adding a test agent; subjecting cultured cells to protease digestion; exposing cells to appropriate polymerase, dNTPs and primers. . . enzyme; exposing the cells to an appropriate substrate for the reporter enzyme; and determining the presence of cryptic form of ***Chlamydia*** by visualizing the amplified DNA encoding a chlamydial

protein, such as MOMP.

DETD . . . susceptibility test can be used to evaluate the status of a human or animal undergoing therapy for the management of ***Chlamydia*** infection. For example, a biological material is isolated from the human or animal to undergo combination therapy. The biological material is treated such that the ***Chlamydia*** is isolated therefrom. This chlamydial isolate is allowed to infect ***Chlamydia*** free cells. These infected cells are then exposed to the combination of agents being used in the individual undergoing combination. . .

DETD . . . method has revealed, for example, that antimicrobial therapy with the triple agents, INH, metronidazole and penicillamine, can completely eradicate C. ***pneumoniae*** from infected mice in four months. Moreover, following complete eradication of chlamydiae, multiple attempts to reinfect these cured mice via. . .

DETD . . . of determining the presence of cryptic chlamydial infections in an animal or cell culture is to expose the culture to ***chlamydia*** -stimulating compounds. Such compounds include (but are not limited to) cycloheximide, corticosteriods (such as prednisone) and other compounds which are known. . .

DETD Antichlamydial Therapy Directed Toward the Initial Stage of ***Chlamydia*** Infection

DETD . . . and electron transfer proteins, as well as nitroreductases. Based upon this, it has been discovered that the initial phase of ***Chlamydia*** infection is susceptible to the antimicrobial effects of nitroimidazoles, nitrofurans and other agents directed against anaerobic metabolism in bacteria.

DETD . . . including ribosomes, DNA and RNA. Nitroimidazoles and nitrofurans currently are not considered to possess antimicrobial activity against members of the ***Chlamydia*** species. This lack of antimicrobial activity, however, is due to the fact that conventional susceptibility testing methods only test for effect on the replicating form of ***Chlamydia*** species.

DETD . . . an agent, such that the modification results in an agent having similar or increased, but not significantly decreased, effectiveness against ***Chlamydia*** , compared to the effectiveness of the parent agent from which the analog or derivative is obtained. This comparison can be. . .

DETD Novel Antichlamydial Therapy Directed Toward the Replicating and Cryptic Stationary Phases of ***Chlamydia*** Infection

DETD A unique class of antichlarnydial agents that is effective against the replicating and cryptic stationary phases of ***Chlamydia*** (and possibly against some other stages of the cryptic phase) have been identified using the susceptibility tests described herein. This. . . available susceptability testing methodologies, it has been discovered that these agents, in combination with other antibiotics, are particularly effective against ***Chlamydia*** . It is believed that the isonicotinic acid congeners target the constitutive production of catalase and peroxidase, which is a characteristic of microorganisms, such as mycobacteria, that infect monocytes and macrophages. ***Chlamydia*** can also successfully infect monocytes and macrophages.

DETD Using INH to eradicate ***Chlamydia*** from macrophages and monocytes subsequently assists these cells in their role of fighting infection. However, these agents appear to be. . .

DETD . . . and its congeners can be used to clear infection from monocytes and/or macrophages. When monocytes and macrophages are infected by ***Chlamydia*** , they become debilitated and cannot properly or effectively fight infection. It is believed that, if the chlamydial infection, per se,. . . one aspect of the invention provides a specific method for reempowering monocytes or macrophages that have been compromised by a ***Chlamydia*** infection and, in turn, comprise treating the infection in other sites. Such compromised macrophages or monocytes can be activated by. . .

DETD Therapy Directed Toward Elementary Bodies of ***Chlamydia***

DETD . . . discovered that adverse conditions, such as limited nutrients, antimicrobial agents, and the host immune response, produce a stringent response in ***Chlamydia*** . Such adverse conditions are known to induce stringent responses in other microorganisms (C. W. Stratton, In: Antibiotics in Laboratory Medicine,. . . Fourth Edition. Lorian V (ed) Williams & Wilkins, Baltimore, pp 579-603 (1996)) and not surprisingly induce a stringent response in ***Chlamydia*** . This stringent response in ***Chlamydia*** alters the morphological state of the intracellular microorganism and creates dormant forms, including the intracellular EB, which then can cryptically. . . the extracellular milieu. Thus, it is necessary to utilize a combination of agents directed toward the various life stages of ***Chlamydia*** and, in particular, against the elementary body for successful management of infection.

DETD . . . these metabolically-inactive EBs escape the action of current antichlamydial therapy which is directed only against the replicating intracellular forms of ***Chlamydia*** . The presence of infectious extracellular EBs after the completion of short term. anti-replicating phase therapy for chlamydial infections has been. . .

DETD . . . is also believed that persistance of chlamydial infections, in part, may be due to the presence of cryptic forms of ***Chlamydia*** within the cells. This cryptic intracellular chlamydial form apparently can be activated by certain host factors such as cortisone (Yang. . . Infection and Immunity, 39:655-658 (1983); and Malinverni et al., The Journal of Infectious Diseases, 172:593-594 (1995)). Antichlamydial therapy for chronic ***Chlamydia*** infections must be continued until any intracellular EBs or other intracellular cryptic forms have been activated and extracellular EBs have. . .

DETD . . . their respective hosts by reducing disulfide bonds which maintain the integrity of the outer membrane proteins of the EBs. For ***Chlamydia*** , disruption of the outer membrane proteins of EBs thereby initiates the transition of the EB form to the RB form.. . .

DETD Currently Recognized Agents Active Against ***Chlamydia*** Replication

DETD . . . they begin to utilize active transcription of chlamydial DNA and translation of the resulting mRNA. As such, these forms of ***Chlamydia*** are susceptible to currently used antimicrobial agents. The antichlamydial effectiveness of these agents can be significantly improved by using them in combination with other agents directed at different stages of ***Chlamydia*** life cycle, as discussed herein.

DETD

TABLE 5

Agents Effective Against the Replicating

Phase of ***Chlamydia***
Drug Class Examples Preferred

Quinolones/ Ofloxacin Levofloxacin
Fluoroquinolones Levofloxacin
Trovafloxacin
Sparfloxacin
Norfloxacin
Lomefloxacin
Cinoxacin
Enoxacin
Nalidixic Acid
Fleroxacin
Ciprofloxacin

. . .

DETD All members of the ***Chlamydia*** species, including C. ***pneumoniae*** , are considered to be inhibited, and some killed, by the use of a single agent selected from currently used antimicrobial agents such as those described above. However, using the new susceptability test, the inventors have found complete eradication of ***Chlamydia*** cannot be achieved by the use of any one of these agents alone because none are efficacious against all phases of the ***Chlamydia*** life cycle and appear to induce a stringent response in ***Chlamydia*** causing the replicating phase to transform into cryptic forms. This results in a persistent infection in vivo or in vitro. . . DNA. Nevertheless, one or more of these currently used agents, or a new agent directed against the replicating phase of ***Chlamydia*** , should be included as one of the chlamydial agents in a combination therapy in order to slow or halt the. . .

DETD . . . attempting to manage or eradicate a systemic infection, it is critical to target multiple phases in the life cycle of ***Chlamydia*** , otherwise viable ***Chlamydia*** in the untargeted phases will remain after therapy and result in continued, chronic infection. This fundamental insight is at the. . .

DETD 2. Evaluate the relative importance of targeting each particular phase in eradicating reservoirs of ***Chlamydia*** from the host organism. For example, the life-cycle stages listed in step 1 can be prioritized based on the following. . .

DETD . . . reproduction cycle seen in cycloheximide-treated eukaryotic cells is an artifact of an atypical, cell culture environment designed primarily to propagate ***Chlamydia*** .

DETD c. The transition phases represent only a small portion of ***Chlamydia*** in chronic infections.

DETD 3. Identify "targets" for each phase of the selected life cycle phases. A target is an attribute of ***Chlamydia*** which is vulnerable during a particular life cycle phase. For example, the disulfide bonds in MOMP are a target during. . .

DETD . . . new theoretical targets within Chlarnydia are identified, or new drugs are developed which attack currently known or new targets within ***Chlamydia*** . For example, the phases of the life cycle could be further sub-classified based on the type of host cell the. .

DETD . . . a Combination Therapy
Potentially Constituitive DNA- Ribosomes
vulnerable production of dependent involved in

attributes of Disulfide Non-oxidative peroxidases and RNA Folic acid protein ***Chlamydia*** : bonds metabolism catalyses Topoisomerases polymerase pathway synthesis . . .

Relative
Phase in Chlamydial Impor-
Life Cycle Theoretical Targets tance
EB (Extracellular or. . .
DETD An association has been discovered between chronic ***Chlamydia*** infection of body fluids and/or tissues with several disease syndromes of previously unknown etiology in humans which respond to unique. . . neural-mediated hypotension); Pyoderma Gangrenosum (PG), Chronic Fatigue (CF) and Chronic Fatigue Syndrome (CFS). Other diseases are under investigation. Correlation between ***Chlamydia*** infection and these diseases has only recently been established as a result of the diagnostic methodologies and combination therapies described. . .
DETD Based on this evidence, published evidence of an association between atherosclerosis and ***Chlamydia*** (Grupta el al, Circulation 96:404-407 (1997)), and an understanding of the impact ***Chlamydia*** infections have on infected cells and the immune systems, the inventors have discovered a connection between ***Chlamydia*** and a broad set of inflammatory, autoimmune, and immune deficiency diseases. Thus, the invention describes methods for diagnosing and/or treating disease associated with ***Chlamydia*** infection, such as autoimmune diseases, inflammatory diseases and diseases that occur in immunocompromised individuals by diagnosing and/or treating the ***Chlamydia*** infection in an individual in need thereof, using any of the assays or therapies described herein. Progress of the treatment can be evaluated serologically, to determine the presence or absence of ***Chlamydia*** using for example the diagnostic methods provided herein. and this value can be compared to serological values taken earlier in. . . then alternate compounds should be substituted in order to achieve the lower antibody titers that demonstrate specific susceptability of the ***Chlamydia*** to the new regimen. A replacement or substitution of one agent with another agent that is effective against the same life stage of ***Chlamydia*** is desirable.
DETD . . . be used for the treatment of acute and chronic immune and autoimmune diseases when patients are demonstrated to have a ***Chlamydia*** load by the diagnostic procedures described herein which diseases include, but are not limited to, chronic hepatitis, systemic lupus erythematosus,. . .
DETD . . . peripheral neuropathy, chronic or recurrent sore throat, laryngitis, tracheobronchitis. chronic vascular headaches (including migraines, cluster headaches and tension headaches) and ***pneumonia*** when demonstrated to be pathogenically related to ***Chlamydia*** infection.
DETD Treatable disorders when associated with ***Chlamydia*** infection also include, but are not limited to, neurodegenerative diseases, including, but not limited to, demyelinating diseases, such as multiple. . .
DETD . . . the diseases indicated were observed and are reported in Example 5. The data provides evidence to establish that treatment of ***Chlamydia*** infection results in the serological and physical improvement of a disease state in the patient undergoing combination

therapy. These observations. . .

DETD Other Diseases of Unknown Etiology with New Evidence for a ***Chlamydia*** ***Pneumoniae*** Etiology

DETD Both C. ***trachomatis*** and C. psittaci exhibit a protean disease complex dependent on different serovars. One known basis for this diversity to date is the amino acid sequence of the MOMP. FIG. 1 shows a sequence alignment of various ***Chlamydia*** MOMPs. Note that the size and sequence are relatively homologous except for the four variable regions that are responsible for the serovar (serotype) basis of classification. Further, it has been discovered that C. ***pneumoniae*** infects blood vessel endothelial cells from which EBs are released in the blood stream. In addition, macrophages are known targets for C. ***pneumoniae*** and may serve as reservoirs and provide an additional mechanism of transmission. C. ***pneumoniae*** is thus able to spread throughout the human body, establishing infection in multiple sites and in multiple organ systems. Infected. . .

DETD . . . intended to embrace both humans and animals. Virtually all rabbits and mice tested to date have PCR signals for C. ***pneumoniae*** . They can be used as appropriate animal models for treatment using specific combination antibiotics to improve therapy. (Banks et al.,. . .

DETD Coupled with these developments are the recently developed rabbit models of coronary artery disease, where rabbits exposed to C. ***pneumoniae*** subsequently develop arterial plaques similar to humans (Fong et al., J. Clin. Microbiol. 35:48-52 (1997)). Most recently, a study at. . . George's Hospital in London found that roughly 3/4 of 213 heart attach victims have significant levels of antibodies to C. ***pneumoniae*** antibody and that those that have such antibodies achieve significantly lower rates of further adverse cardiac events when treated with. . .

DETD . . . also been introduced based on the report that Vitamin C (ascorbic acid) at moderate intracellular concentrations stimulates replication of C. ***trachomatis*** (Wang el al., J. Clin. Micro. 30:2551-2554 (1992)) as well as its potential effect on biofilm charge and infectivity of. . .

DETD ***Chlamydia*** is a parasite of normal energy production in infected eukaryotic cells. As a result, host cells have insufficient energy available. . . cell mitochondria to attempt to synthesize certain critical enzymes involved in energy production in order to increase energy production. Because ***Chlamydia*** also prevents this synthesis from completing, these enzyme's precursors, called porphyrins, build up in cell and often escape into the. . .

DETD . . . this secondary form of porphyria, a unique approach for the diagnosis and treatment of obligatory and secondary disorders caused by ***Chlamydia*** infections has been developed. The adjunctive therapy described herein can be used in combination with the appropriate antimicrobial therapy required. . .

DETD . . . Y., Microbiological Reviews, 42:247-306 (1978); McClairty, G., Microbiology, 2:157-164(1994)). The transition of elementary bodies (EBs) to reticulate bodies (RBs) for ***Chlamydia*** species requires the presence of functioning mitochondria in the infected cell as well as the production by the host cell. . .

DETD B. ***Chlamydia*** and Secondary Porphyria

DETD . . . step in the biosynthesis of heme as it catalyses the oxidative entry of coproporphyrinogen into the mitochondria matrix as

protoporphyrin; ***Chlamydia*** interfere with this step by reducing electron transfer in the host cell. When coproporhyrinogen is unable to return to the. . .

DETD Depletion of host cell energy by the intracellular infection with ***Chlamydia*** species causes additional energy-related complications. As fewer electrons are available to move through the electron transport chain of the host. . .

DETD . . . the classical manifestations of hereditary porphyria. As the chlamydial-infected host cells lyse, as happens in the normal life cycle of ***Chlamydia*** , the intracellular porphyrins are released and result in a secondary porphyria. Moreover, when the chlamydial infection involves hepatic cells, the. . . is a heme-based enzyme. Hence, the biosynthesis of heme in the liver becomes increased. When hepatic cells are infected with ***Chlamydia*** species, the decreased energy in the host cell does not allow heme biosynthesis to go to completion and porphyrins in the liver/entero-hepatic circulation are increased. It also has been noted that any host cell infected with ***Chlamydia*** species has an increased amount of intracellular porphyrins that are released when antimicrobial agents kill the microorganism.

DETD . . . clearly is of paramount importance in dealing with chronic systemic chlamydial infections as are seen with intravascular infections caused by ***Chlamydia*** ***pneumoniae*** .

DETD . . . (Kordac V., Neoplasma, 19:135-139 (1972); Lithner et al., Acta Medica Scandanavia, 215:271-274 (1984)). Of particular interest is that infection with ***Chlamydia*** ***pneumoniae*** has been associated with lung cancer (Cerutti P A., Science, 227:375-381 (1985)).

DETD . . . foregoing discussion of the etiology of porphyria, one aspect of the invention pertains to methods for differentiating porphyria caused by ***Chlamydia*** from that caused by a latent genetic disorder in an individual. The method comprises treating infection by ***Chlamydia*** at all stages of its life cycle, using the therapies described in detail elsewhere in this disclosure, and then assessing. . . symptoms of porphyria (e.g., biochemical, enzymatic or physical manifestation) are indicative that the porphyria is a secondary porphyria caused by ***Chlamydia*** .

DETD . . . is suggestive of a non-genetic porphyria, such as chlamydially induced secondary porphyria. For example, in one embodiment, porphyria caused by ***Chlamydia*** in an individual having symptoms associated therewith can be diagnosed by determining the presence and/or amount of obligatory enzymes in. . .

DETD As discussed above, some patients having a ***Chlamydia*** -induced porphyria do not have abnormal levels of heme precursors. For those patients it may be appropriate to determine the presence of ***Chlamydia*** as well as porphyrins in the individual. The presence of both the pathogen and porphyrins (e.g., determined by ELISA assay. . .

DETD . . . well as Vitamin B12 (cobalamin), which is molecularly similar to these metabolites, in patients with active systemic infection with C. ***pneumoniae*** . The antibodies are primarily IgM; this is similar to the antibody responses to the MOMP of C. ***pneumoniae*** in severely symptomatic patients. Example 8 illustrates titers in symptomatic patients with systemic C. ***pneumoniae*** infections. The presence of antibodies to Vitamin B12 may have functional significance by decreasing the amount of bioavilable Vitamin B12. Thus, a ***Chlamydia*** infection may cause a previously unrecognized

secondary Vitamin B12 deficiency. Administration (e.g., intramuscular) of large quantities of Vitamin B12 (1000. . .

DETD Treatment of ***Chlamydia*** infection may exaserbate secondary porphyria by increasing the metabolism of cryptic ***Chlamydia*** or by accelerating the death of infected cells with elevated intracellular porphyrin levels.

DETD To reduce severe porphyric attacks during therapy for chronic ***Chlamydia*** infections, the use of hemodialysis. plasmapheresis, chelating agents and/or intravenous hematin may be needed. Any one of these or a. . .

DETD . . . nutritional formulations including beverages and foods such as nutritional bar, for the management of non-genetic, secondary porphyria caused by a ***Chlamydia*** infection. Alternatively, a combination of vitamins and antioxidants can be co-packed in a pack or kit as described elsewhere herein. . .

DETD . . . to ameliorate conditions/symptoms associated with the disease states described above, when the disease is onset or aggravated by infection by ***Chlamydia*** . The agents of this invention can be administered to animals including, but not limited to, fish, amphibians, reptiles, avians and. . .

DETD . . . thereof. The agents can also be used for the manufacture of a medicament for therapy of a disease associated with ***chlamydia*** infection, such as autoimmune disease, inflammatory disease, immunodeficiency disease.

DETD Polymerase Chain Reaction (PCR) for the Full Length MOMP Gene of C. ***Pneumoniae*** and other Species of ***Chlamydia*** (Diagnostic)

DETD . . . which on repeated assay without reducing agents, yields a negative PCR signal for the 1.2 kB MOMP gene of C. ***pneumoniae*** . Analysis on agarose gel with ethidium bromide visualization under UV light.

DETD . . . signals using the preferred primers which amplify the full length MOMP gene suggests that mutations in these regions of C. ***pneumoniae*** is rare. Standard conditions for this gene product in a 50-.mu.l volume is 35 cycles with 1 second ramp times. . .

DETD This procedure identifies individual cells containing RB and cryptic forms of C. ***pneumoniae*** . Cultured cells are adhered to glass slides with formalin, or formalin fixed tissue sections embedded in paraffin are adhered to. . .

DETD The full length MOMP gene of C. ***pneumoniae*** was directionally cloned into the pET expression plasmid at the NCOI and NOTI restriction sites using primers to introduce these. . .

DETD . . . length expressed recombinant fusion protein or the modified MOMP following endopeptidase cleavage can be used as the antigen for a ***Chlamydia*** species ELISA. Other expression systems in E. coli or Baculovirus can be used for synthesis of the MOMP protein as. . .

DETD The recombinant MOMP-based ELISA described above provides a sensitive method for the quantitation of immunoglobulins against the ***Chlamydia*** genus in serum, plasma, CSF, and other body fluids. In order to provide ELISA assays that are species- and potentially strain-specific for the various ***Chlamydia*** , two regions in the MOMP have been identified which show minimal amino acid sequence homologies and which are predicted by. . . parallels that described above for the recombinant MOMP-based ELISA. In addition, a highly antigenic domain (FIG. 6) common to all ***Chlamydia*** has been identified and was developed as an alternative genus-specific ELISA for

the Chiamydia. Immunization of rabbits has verified the. . .
DETD
TABLE 9

Antigenic Responses To Peptides From 4 Species of ***Chlamydia***
Identified By Hydrophilicity And Peptide Movement
As Highly Antigenic
Chlamydia Titer.sup.a
Species Peptide.sup.b Pre Post

C. ***pneumoniae*** 90-105 100 >3200
C. ***trachomatis*** L2 91-106 800 >3200
C. psittaci 92-106 400 >3200
C. ***trachomatis*** (mouse) 89-105 0 >3200
C. ***pneumoniae*** 158-171 25 >3200
C. ***trachomatis*** L2 159-175 200 >3200
C. psittaci 160-172 100 >3200
C. ***trachomatis*** (mouse) 158-171 800 >3200
C. ***pneumoniae*** 342-354 200 >3200
C. ***trachomatis*** L2 342-354 100 >3200
C. psittaci ND.sup.c
C. ***trachomatis*** (mouse) ND.sup.c

.sup.aReciprocal titer
.sup.bImmunogenic peptide and ELISA antigen of specific amino acid sequence against the indicated pre-immunization. . .
DETD Table 10 illustrates reciprocal titers of a polyclonal and monoclonal antibody against C. ***trachomatis*** cross-reactive against C. ***pneumoniae*** peptide encompassing amino acids 342-354 and a recombinant full length MOMP from C. ***pneumoniae*** .
DETD
TABLE 10

Reciprocal titers of a polyclonal and a monoclonal antibody
against C. ***trachomatis*** cross-reactive against C. ***pneumoniae***
peptide encompassing amino acids 342-354 and a recombinant
full length MOMP from C. ***pneumoniae***
Titer.sup.a
Antigen Polyclonal Ab.sup.b Monoclonal Ab.sup.c

CPN Momp.sup.d 400 0
CPN 90-105.sup.e 50 0
CPN 158-171.sup.f 50 0
CPN 342-354.sup.g >3200 1600

.sup.aReciprocal titer
.sup.bPolyclonal goat Ab from Chemicon Inc. against MOMP of C. ***trachomatis***
.sup.cMonoclonal Ab (ICN, Inc.) against MOMP of C. ***trachomatis***
.sup.dC. ***pneumoniae*** recombinant MOMP
.sup.eAmino acid peptide 90-105 of C. ***pneumoniae***
.sup.fAmino acid peptide 158-171 of C. ***pneumoniae***
.sup.gAmino acid peptide 342-354 of C. ***pneumoniae***
DETD C. ***pneumoniae*** EBs were grown in primary human umbilical vein endothelial cells (HuEVEC; early passage), HeLa 199, or a suitable

alternative in. . .
DETD Western blots were prepared by SDS-PAGE of C. ***pneumoniae*** EBs (non-formalin fixed) harvested from infected HuEVEC or HeLa cell lysates, electrophoresed under standard SDS-PAGE conditions, and transferred to nitrocellulose. . .
DETD In Vitro Antimicrobial Susceptability Testing for C. ***Pneumoniae***
DETD Tissue culture cells containing cryptic phase C. ***pneumoniae*** (H-292, HeLa, HEL, HuEVEC, McCoy, etc.) are plated at subconfluency in a 96-well microtiter plate (flasks or plates or other. . .
DETD . . . at 1 .mu.g/ml failed to clear HeLa cells in culture of a detectable PCR signal for the MOMP gene of ***Chlamydia*** ***pneumoniae*** . In contrast, triple agents consisting of isoniazid (INH), metronidazole, and penicillamine (1 .mu.g/ml each) resulted in no detectable PCR signal. . .
DETD
TABLE 11

Susceptibility to Antibiotics for Cryptic C. ***pneumoniae***
Cultured in HeLa Cells.sup.a
Antibiotic Conc (.mu.g/ml) PCR.sup.b

Ofloxacin 1 positive
Clarithromycin 1 positive
INH 1 positive
Metronidazole 1 positive
. . .
DETD
TABLE 12

Susceptibility to Antibiotics by PCR for
Cryptic ***Chlamydia*** ***pneumoniae*** Cultured in HeLa Cells.sup.1
Phase of the Chlamydial Life Cycle
EB (Extracellular EB->RB Stationary Phase RB RB->EB Transition Concentration PCR PCR. . .
DETD . . . typical responses to combination antibiotic therapy in a variety of patients with diagnostic evidence of an active infection by C. ***pneumoniae*** . Unlike typical immune responses to infection with infectious agents, most of the included patients have not only detectable IgM titers. . . the IgM titers generally fall, with a rise in IgG titer (as expected). Current methods of detecting antibodies against C. ***pneumoniae*** (Indirect immunofluoresence, MIF) are incapable of accurately identifying high IgM titers against C. ***pneumoniae*** . Moreover, current procedures are genus specific and not species specific as are peptide-based ELISAs. With clearing of the pathogen, the. . .
DETD . . . months with an EDSS = 8.0
(triplegic plus speech and swallowing impairments). A positive CSF PCR and culture for C. ***pneumoniae*** led to treatment with combination antibiotics. The
patient improved on all spheres of neurologic function over the following six
months.. . . legs. Over 5 months his EDSS score worsened from 7.0 to 8.0. His CSF was positive by PCR for C. ***pneumoniae*** and he was placed on combination antibiotics. Over the next six months he gradually improved in his

balance, coordination and. . . to
response to corticosteroids on two successive occasions. Five months later, his
EDSS score was 7.5. Following a positive C. ***pneumoniae*** PCR he was placed on
combination antibiotics. He has gradually gained strength in his lower
extremities and five months later. . . progressive MS with recent
progressive bulbar symptoms, axtaxia, and paraplegia (EDSS = 8.5). PCR for the
MOMP gene of C. ***pneumoniae*** in the CSF was positive. She was placed on
combination antibiotics with no further progression of symptoms for the last. . . ulcers improved again.
TW PG Severe PG, initiated after a chemical burn in 1991, but with PCR negative
serology for C. ***pneumoniae*** . Patient did not initially respond to combination
antibiotic therapy. A positive biopsy culture for C. ***pneumoniae*** resulted in the
recent re-institution of combination antibiotics. However, after no
improvement, patient went off therapy.
AM IBD Row 5 This. . . the
colectomy, the patient experienced neurologic symptoms, fatigue, myalgias,
arthralgias, and an acneoform skin rash. Serology was performed for C. ***pneumoniae*** and was positive with an IgM of 1:3200, IgG 1:400 and PCR positive.
Therapy with combination antibiotics was initiated. After. . . resolution
of her proctitis on visual exam.
NM CFS Vanderbilt University initial patient that resulted in our first association of C.
pneumoniae , initially complained of the insidious onset of debilitating fatigue.
This was associated with a severe cognitive dysfunction that disrupted his. . . Infectious Disease Clinic at
Vanderbilt no definitive or presumptive diagnosis could be made. A subsequent
high antibody titer against C. ***pneumoniae*** led to standard anti-chlamydial
antibiotic therapy over a three month period with gradual disappearance of
fatigue and cognition symptoms. On. . . developed acute anxiety attacks relieved by anti-
porphyrin therapy.
WM CF Row 7 CF following acute stress. Pre-illness serum negative for anti-
Chlamydia
pneumoniae antibodies which peaked six weeks following stress. Pre-illness PCR
was weak positive that became strongly positive. On combination antibiotic
therapy. . .
DETD
TABLE 14

Examples of Secondary Porphyria in Patients with Systemic infections caused by C. ***pneumoniae*** .sup.a
Enzymes of Heme
biosynthesis.sup.b

Patient ALA PBG Elevated Fecal Porphyrins (24 hr) Elevated Urinary Porphyrins (24 hr)
ID synthase deaminase Porphyrin. . .
DETD A set of mice were tested for infection with C. ***pneumoniae*** . Of 10 mice tested, 8 (80%) were PCR positive for C. ***pneumoniae*** . The mice were then placed on triple-antibiotic therapy: Amoxicillin, Metronidazole and fNH at 50 .mu.g/ml each in their water. Based. . .
DETD Patents with systemic infections caused by C. ***pneumoniae*** were evaluated for secondary porphyria. The presence of enzymes (i.e., .DELTA.-ALA synthase and PBG deaminase) for heme biosynthesis were determined. . .
DETD
TABLE 14

Examples of Secondary Porphyria in Patients with Systemic infections caused by C. ***pneumoniae*** .sup.a
Enzymes of Heme
biosynthesis.sup.b
Patient ALA PBG Elevated Fecal Porphyrins (24 hr) Elevated Urinary Porphyrins (24 hr)
ID synthase deaminase Porphyrin. . .
DETD Patients with systemic infections caused by C. ***pneumoniae*** were tested for the presence of antibodies to porphyrin ring structures (i.e., vitamin B12, coproporphyrinogen-III, protoporphyrin, porphyrobelinigen and .DELTA.-ALA). IgM. . .
DETD
TABLE 15

Examples of Antibody Titers.sup.a- to Porphyrin Ring Structures in Patients with Systemic
infections caused by C. ***pneumoniae***
Patient B12 Copro III Protoporphyrin Porphyrobelinigen --ALA
ID IgM IgG IgM IgG IgM IgG IgM IgG IgM IgG

KRH 1:640 1:160 1:640 1:160. . .
DETD . . . Ser Lys Leu
1 5 10 15
Val Pro
SEQUENCE CHARACTERISTICS:
LENGTH: 14 amino acids
TYPE: amino acid
STRANDEDNESS: single
TOPOLOGY: linear
MOLECULE TYPE: peptide
SEQUENCE: 100
Cys ***Ile*** ***Gly*** ***Leu*** ***Ala***
Gly ***Thr*** ***Asp*** ***Phe*** ***Ala***
Asn ***Gln*** ***Arg*** ***Pro***
1 5 10
SEQUENCE CHARACTERISTICS:
LENGTH: 13 amino acids
TYPE: amino acid
STRANDEDNESS: single
TOPOLOGY: linear
MOLECULE TYPE: peptide

SEQUENCE: 101
Cys Gln Ile Asn Lys Phe. . .